Supporting Information

Discovery of Isoindoline Amide Derivatives as Potent and Orally Bioavailable ADAMTS-4/5 Inhibitor for the Treatment of Osteoarthritis

Peng Zhao[1]*#, Dong Liu[1#], Chunying Song[1*], Di Li[1], Xinzhu Zhang[1], Ivana Horecny[1], Fengqi Zhang[1], Yuna Yan[2], Linghang Zhuang[1], Jing Li[1], Suxing Liu[1], Yuchang Mao[2], Jun Feng[2], Jian Liu[1], Weikang Tao[2]

[1]Eternity Bioscience Inc., 6 Cedarbrook Drive, Cranbury, NJ 08512, USA.

[2]Shanghai Hengrui Pharmaceutical Co. Ltd., 279 Wenjing Road, Shanghai 200245, China.

*Email: pengebi.zhao@hengrui.com

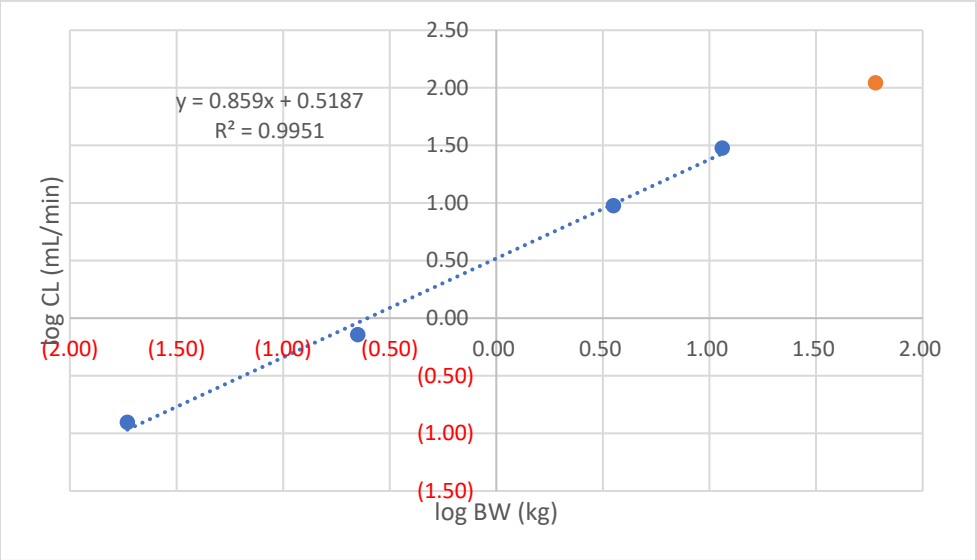
*Email: song_cy@hotmail.com

#These authors contributed equally to this work and share the first authorship.

Content

Table S1. Human PK prediction of compound 18

Species	$t_{1/2}$	iv CL	iv Vd	Body Weight (BW)	iv CL* BW	F%
	(h)	(mL/min/kg)	(mL/kg)	(kg)	(mL/min)	
Mouse	1.7	6.7	976	0.019	0.13	88
Rat	3.2	3.2	869	0.224	0.72	51
Dog	6.1	2.6	1399	11.470	30.05	121
Monkey	7.3	2.7	1635	3.540	9.52	67
Human (predicted)	7.4	1.9	1220	60	111	82



Predicted human CL is marked in orange.

Molecular modeling

The software package Schrödinger (version: 2021-2) was used for our modeling study. The crystal structure (PDB:6YJM) binding with GLPG1972 was prepared with the Protein Preparation Wizard (PrepWizard) in Maestro prior to docking. LigPrep was used with default settings (OPLS4 force field) to prepare the compounds. The default Glide options for grid generation were employed. Compounds were docked into the binding pocket using Glide with Glide SP scoring function. The top one pose for each compound scored by Glide SP scoring function was retained. The figures were generated by MOE (MOLECULAR OPERATING ENVIRONMENT 2020.09).

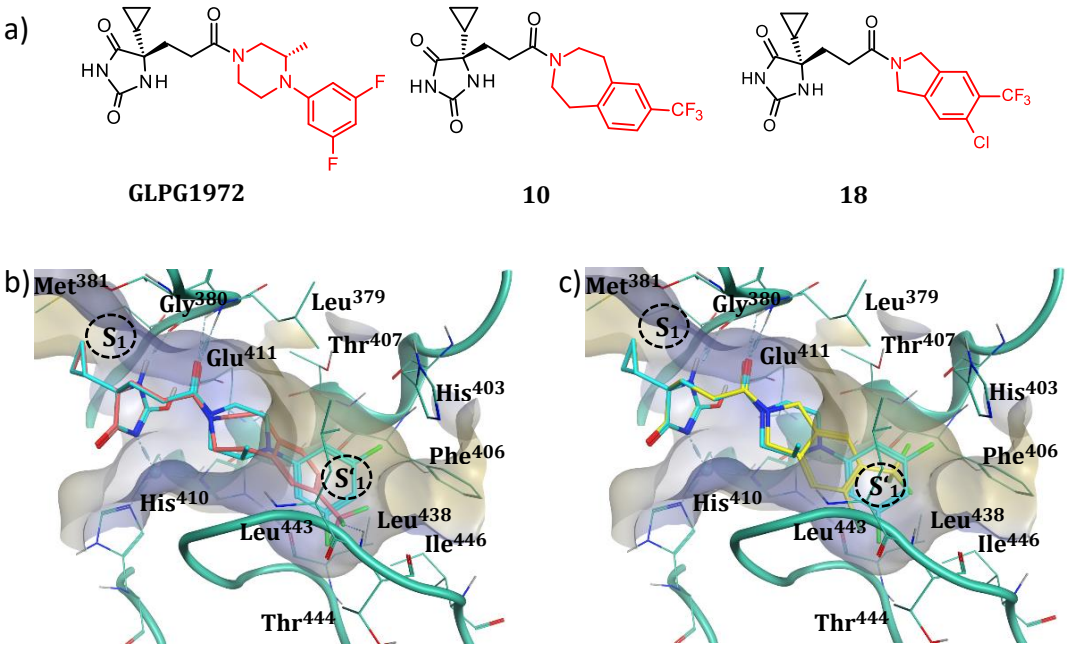


Figure S1. Docking results of 10 and 18 based on PDB: 6YJM. a) 2D structure of GLPG1972, molecule **10** and **18**; b) Binding mode of GLPG1972 (colored in cyan; stick representation) and **10** (colored in orange; stick representation); c) Binding mode of GLPG1972 (colored in cyan; stick representation) and **18** (colored in yellow; stick representation). Co-crystal structure of GLPG1972 in ADAMTS-5 (PDB ID: 6YJM). **10** and **18** were docked into the same binding site of GLPG1972. The hydrogen bonds between protein residues and ligands are colored in light blue. The surface is colored by lipophilicity of the residue (from blue for hydrophilic to brown

for lipophilic).

Based on the reported crystal structure of ADAMTS-5 complexed with GLPG1972 (PDB# 6YJM), Compounds **10** and **18** were docked into the GLPG1972 binding pocket using Glide with SP (standard precision) mode (**Figure S1**).

Both **10** and **18** shared the same left-side fragment with GLPG1972 and made the same interaction with left-side S_1 pocket. The hydantoin ring adopted a tautomeric form to coordinate with the zinc ion through the nitrogen atom of the N-acyl amide and form H-bond with Glu411. Another H-bond was formed between the hydantoin amide nitrogen and Gly380. The cyclopropyl group made hydrophobic interaction with Met381 in this S_1 pocket. For the right-side fragment, azepane of **10** and pyrrolidine of **18** occupied the position of the piperazine ring in GLPG1972 to make hydrophobic interactions with His410, Leu443 and Leu379. Substituted phenyl ring of **10** and **18** embedded in the S'_1 pocket with hydrophobic interactions with His403, Phe406, Leu438, Ile446, Leu443, His410 and Thr444. The phenyl rings in **10** and **18** both were closer to imidazole of His410 than GLPG1972 to be helpful for π-π interaction (4.52Å *vs* 5.66Å and 4.02Å *vs* 5.66Å, respectively; the distance was measured between the center of benzene and imidazole ring). Additionally, the 2.74Å distance between the -F of $-CF_3$ in **10** and -NH of Thr444 could potentially form polar interaction for better affinity.

To occupy the hydrophobic pocket S'_1, careful optimization of both fragment volume and hydrophobicity of R^1 and R^2 substituents on isoindoline core was required to achieve the best potency. Single -H (molecule **11**) or -F (molecule **11**) were apparently too small for this pocket based on the modeling result and the -CN in **13** was unfavorable for hydrophobic interaction. This S'_1 pocket sensitivity can also rationalize the previous findings on ADAMTS-5 potency order with substituent group size in **14**, **17**, **18** and **20** (**Table 2**).

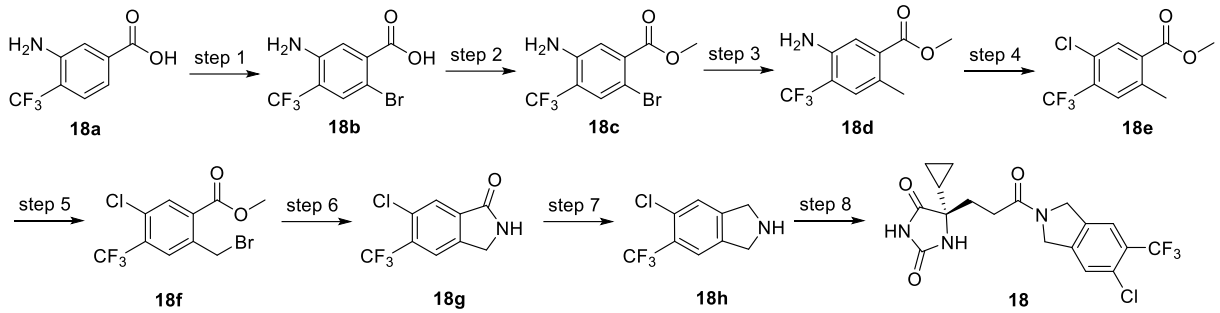
Experimental section

General information

All solvents and reagents were commercially available and used without further purification. Unless otherwise stated, the reactions were carried out under nitrogen atmosphere and the reaction temperature refers to room temperature with the range between 20 °C to 30 °C. The reaction process was monitored by LC-MS or thin layer chromatography (TLC). LC/MS (ESI) analyses were performed on a Shimadzu LCMS2020 equipped with a Sunfire C18 (5 μm 50×4.6 mm) column. Analytical thin layer chromatography (TLC) was performed on thin-layer silica gel plates used in TLC were Yantai Huanghai HSGF254 or Qingdao GF254 silica gel plate. The target compounds and intermediates were purified by ISCO CombiFlash rapid preparation instrument and technical grade solvents. Prep-HPLC was performed on Shimadzu LC-20AD, SPD20A with Phenomenex Gemini-NX 5 μm C18 21.2×100 mm column using water/MeOH or water/ CH_3CN elution systems with optional additives, such as HCOOH, TFA. HPLC analyses were performed on an Agilent 1200DAD equipped with a Sunfire C18 (5 μm 150×4.6 mm) column and Shimadzu UFLC equipped with an Xbridge C18 (5 μm 150×4.6 mm) column. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE-300, AVANCE-400 or AVANCE-500. The solvents are deuterated-dimethyl sulfoxide (DMSO-d_6), deuterated-chloroform ($CDCl_3$) and deuterated-methanol (CD_3OD) with tetramethylsilane (TMS) as an internal standard. NMR chemical shifts (δ) are given in 10^{-6} (ppm) and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), brs (broad singlet), or dd (doublet of doublets). Purity of all final target compounds were determined by HPLC and found to be >95%.

Commercially available amine starting material **2a** (CAS#959239-29-0), **3a** (CAS#911064-58-99), **4a** (CAS#496-12-8), **5a** (CAS#4424-20-8), **6a** (CAS# 127168-78-9), **7a** (CAS#90047-53-3), **8a** (CAS#50351-80-9), **9a** (CAS#324558-64-7), **11a** (CAS#57584-71-1), **12a** (CAS#127168-76-7), **13a** (CAS#263888-58-0), **14a** (CAS#342638-03-3) were directly purchased from the vendors. Custom synthesis of amine starting material **10a** and **17a** was provided by Acme Bioscience Inc (3941 E Bayshore Rd, Palo Alto, CA 94303). Amine **1a** was synthesized by the route reported before.[1] Intermediate **Int-1** and **25a**-**27a** were synthesized and chiral separated by SFC following reported procedure.[2]

(*S*)-5-(3-(5-Chloro-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)-5-cyclopropylimidazolidine-2,4-dione (18)



Step 1: 5-Amino-2-bromo-4-(trifluoromethyl)benzoic acid (18b)

To a solution of 3-amino-4-(trifluoromethyl)benzoic acid **18a** (1.0 g, 4.87 mmol) in DMF (20 mL) was added NBS (870 mg, 4.89 mmol). The mixture was stirred at room temperature for 2 h. The reaction mixture was poured into ice water (20 mL) and extracted with EtOAc (20 mL ×2). The combined organic phase was washed with water (20 mL), brine (20 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated to afford crude **18b** (1.0 g, yield 72.22%).

Step 2: Methyl 5-amino-2-bromo-4-(trifluoromethyl)benzoate (18c)

To a solution of **18b** (1.0 g, 3.52 mmol) in MeOH (10 mL) was added H_2SO_4 (18 M, 0.7 mL) dropwise. After the mixture was stirred at 75 ℃ overnight, the mixture was cooled down to room temperature and poured into ice water (20 mL). The mixture was extracted with EtOAc (50 mL). The organic fraction was dried over Na_2SO_4 and filtered. The filtrate was concentrated to afford crude **18c** (1.0 g, yield 95.29%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.57 (s, 1H), 7.21 (s, 1H), 6.11 (brs, 2H), 3.85 (s, 3H).

Step 3: Methyl 5-amino-2-methyl-4-(trifluoromethyl)benzoate (18d)

To a solution of **18c** (1.0 g, 3.36 mmol) in DMF (10 mL) was added $Pd(PPh_3)_4$ (430 mg, 372.11 μmol), K_3PO_4 (2.2 g, 10.36 mmol) and methylboronic acid (1.0 g, 16.71 mmol). After the mixture was stirred at 130 ℃ under N_2 atmosphere overnight, the mixture was cooled down to room temperature and filtered. The filtrate was concentrated, and the residue was purified by silica gel chromatography to afford **40d** (500 mg, yield 63.91%).

LCMS: MS m/z (ESI): 234.1 $[M+H]^+$.

Step 4: Methyl 5-chloro-2-methyl-4-(trifluoromethyl)benzoate (18e)

Concentrated HCl (2 mL) was added to a solution of **18d** (2.0 g, 8.58 mmol) in acetone (20 mL), and the mixture was stirred at room temperature for 20 min. The mixture was cooled to -5-

0 ℃, a solution of $NaNO_2$ (600 mg, 8.70 mmol) in H_2O (2.5 mL) was added dropwise, and the mixture was stirred at an ambient temperature for 30 min. CuCl (849 mg, 8.58 mmol) was added portion wise at 0 ℃, and the mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was poured into 1N HCl (50 mL) and the mixture was extracted with EtOAc. The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography to afford **18e** (1.3g, yield 60.00%).

Step 5: Methyl 2-bromo-5-chloro-4-(trifluoromethyl)benzoate (18f)

To a solution of **18e** (1.3 g, 5.15 mmol) in CCl_4 (20 mL) was added NBS (1.10 g, 6.18 mmol) and AIBN (25 mg, 154.38 μmol). The mixture was heated to 70 ℃ and stirred overnight. The mixture was cooled to room temperature and filtered. The cake was washed with CCl_4, and the filtrate was concentrated in vacuo to give crude **18f** (1.9 g, yield 111.37%).

Step 6: 6-Chloro-5-(trifluoromethyl)isoindolin-1-one (18g)

To a solution of **18f** (1.9 g, 5.73 mmol) in MeOH (10 mL) was added NH_3/MeOH (20 mL) and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated in vacuo. The residue was purified by silica gel chromatography (hexane: EtOAc=1:1) to afford **18g** (920 mg, yield 68.14%).

LCMS: MS m/z (ESI): 236.0 $[M+H]^+$.

Step 7: 5-Chloro-6-(trifluoromethyl)isoindoline (18h)

To a solution of **18g** (570 mg, 2.42 mmol) in THF (5 mL) was added BH_3/THF (1.0M, 12 mL, 12 mmol) and the mixture was stirred at 60 ℃ overnight. The reaction was cooled to room temperature and quenched with methanol. The mixture was adjusted to pH = 1 with 1M HCl. Then the mixture was heated to 45 ℃ and stirred for 30 min. After cooled to room temperature, the mixture was adjusted to pH = 7-8 with 1M NaOH. Water was added and the mixture was extracted with EtOAc. The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified prep-TLC (DCM: MeOH=10:1) to give **18h** (10 mg, yield 1.87%).

^{1}HNMR (400 MHz, DMSO-*d6*): δ 7.78 (s, 1H), 7.65 (s, 1H), 4.16 (br, 2H), 4.14 (br, 2H).

LCMS: MS m/z (ESI): 222.1 $[M+H]^+$.

Step 8: (*S*)-5-(3-(5-Chloro-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)-5-cyclopropylimidazolidine-2,4-dione (18)

To a solution of **18h** (10 mg, 45.12 µmol) in DMF (2 mL) was added TEA (50 µL), **Int-1** (10 mg, 47.12 µmol), and HATU (17 mg, 45.12 µmol). The reaction mixture was stirred at room temperature for 3 h. Water was added, the mixture extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by prep-HPLC to give **18** (5 mg, yield 26.65%).

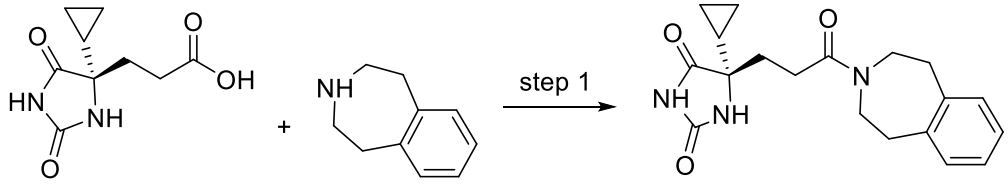
^{1}H NMR (400 MHz, DMSO-d_6): δ 10.63 (s, 1H), 7.90 (s, 1H), 7.76 (s, 1H), 7.75 (s, 1H), 4.85 (d, *J* = 12.4 Hz, 2H), 4.67 (d, *J* = 10.8 Hz, 2H), 2.46-2.22 (m, 2H), 2.03-1.98 (m, 2H), 1.15-1.08 (m, 1H), 0.49-0.31 (m, 3H), 0.15-0.08 (m, 1H).

^{19}F NMR (376.5 MHz, DMSO-d_6): δ -60.86.

^{13}C NMR (400 MHz, DMSO-d_6) δ 178.0, 170.3, 157.3, 144.3, 143.7, 137.6, 137.0, 130.1, 126.8, 123.2, 64.3, 52.0, 51.8, 31.9, 28.4, 16.6, 0.9, -0.5.

HRMS (ESI) m/z for $C_{18}H_{18}ClF_3N_3O_3$ $[M+H]^+$, calculated 416.0989; found 416.0992.

(S)-5-Cyclopropyl-5-[3-oxo-3-(1, 2, 4, 5-tetrahydro-3-benzazepin-3-yl) propyl] imidazolidine-2, 4-dione (5)



To a solution of 2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine **5a** (100 mg, 679.27 µmol) in DMF (4 mL) was added triethylamine (275 mg, 2.72 mmol, 377.67 µL), **Int-1** (173 mg, 815.13 µmol) and HATU (310 mg, 815.13 µmol). The mixture was stirred at room temperature overnight. Water (25 mL) was added, and the reaction mixture extracted with EtOAc (20 mL X2). The combined organic layer was washed with water (40 mL) and brine (40 mL X 2), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The crude product was purified by prep-HPLC to give title compound **5** (11 mg, 4.74%).

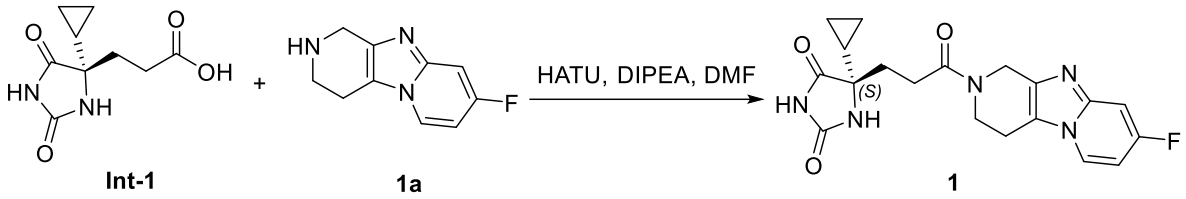
^{1}H NMR (400 MHz, DMSO-d_6): δ 10.62 (s, 1H), 7.72 (s, 1H), 7.15-7.13 (m, 4H), 3.58-3.51 (m, 4H), 2.91-2.90 (m, 2H), 2.82-2.81 (m, 2H), 2.45-2.35 (m, 1H), 2.30-2.25 (m, 1H), δ1.94 (m, 2H), 1.12-1.08 (m, 1H), 0.48-0.46 (m, 1H), 0.40-0.29 (m, 2H), 0.10-0.08 (m, 1H).

^{13}C NMR (400 MHz, DMSO-d_6) δ 178.1, 170.3, 157.3, 141.3, 140.7, 130.1, 130.0, 126.9, 126.7, 64.4, 48.0, 44.7, 37.6, 36.9, 32.6, 27.7, 16.5, 1.0, -0.5.

LCMS: MS m/z (ESI): 342.2 $[M+H]^+$.

Compound **1-4**, **6-14** and **17** were prepared using the foregoing methodology and general procedure described in **5** but substituting with appropriate amines in HATU coupling step.

(S)-5-Cyclopropyl-5-(3-(8-fluoro-3,4-dihydroimidazo[1,2-a:4,5-c']dipyridin-2(1H)-yl)-3-oxopropyl)imidazolidine-2,4-dione (1)

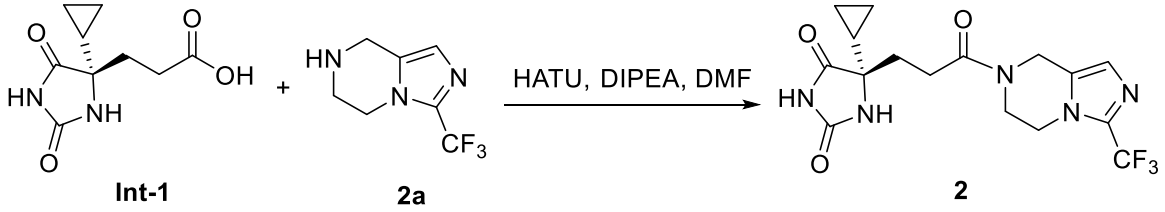


40 mg compound **1** was obatined from 70 mg **Int-1** and 80 mg **1a**, yield 37.8%.

^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.60 (brs, 1H), 8.38-8.33 (m, 1H), 7.74 (d, *J* = 6.4 Hz, 1H), 7.37 (d, *J* = 10.0 Hz, 1H), 7.02-6.96 (m, 1H), 4.63 (s, 2H), 3.92-3.81 (m, 2H), 2.96 (br, 1H), 2.85 (brs, 1H), 2.59-2.33 (m, 2H), 2.04-1.91 (m, 2H), 1.15-1.05 (m, 1H), 0.49-0.27 (m, 3H), 0.14-0.05 (m, 1H).

LCMS: MS m/z (ESI): 386.1 $[M+H]^+$.

(S)-5-Cyclopropyl-5-(3-oxo-3-(3-(trifluoromethyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl)propyl)imidazolidine-2,4-dione (2)

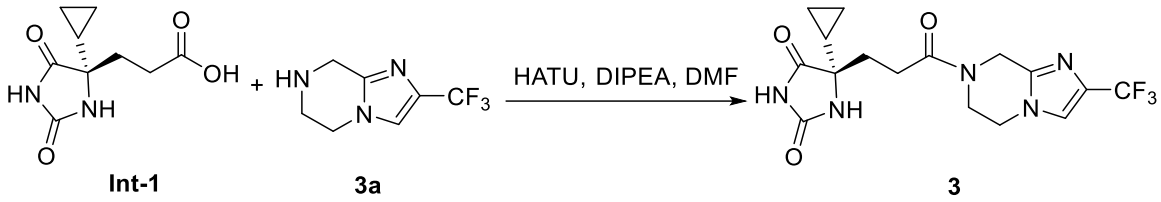


9 mg compound **2** was obtained from 22 mg **Int-1** and 20 mg **2a**, yield 22.4%.

^{1}H NMR (400 MHz, Methanol-d_4) δ 6.90 (s, 1H), 4.78 (s, 2H), 4.21-4.19 (m, 1H), 4.13-4.10 (m, 1H), 3.94-3.84 (m, 2H), 2.52-2.48 (m, 1H), 2.41-2.35 (m, 1H), 2.11-2.00 (m, 2H), 1.14-1.09 (m, 1H), 0.49-0.46 (m, 1H), 0.35-0.20 (m, 3H).

LC-MS: MS m/z (ESI): 386.2 $[M+H]^+$.

(S)-5-Cyclopropyl-5-(3-oxo-3-(2-(trifluoromethyl)-5,6-dihydroimidazo[1,2-a]pyrazin-7(8H)-yl)propyl)imidazolidine-2,4-dione (3)



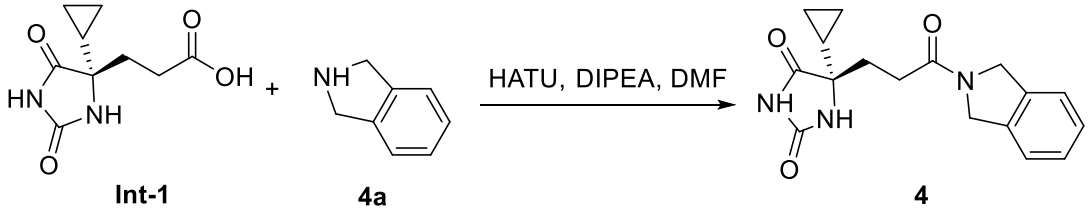
Int-1 3a 3

12 mg compound **3** was obtained from 24 mg **Int-1** and 21 mg **3a**, yield 27.8%.

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.49 (s, 1H), 4.69 (s, 2H), 4.10-3.86 (m, 4H), 2.55-2.49 (m, 1H), 2.42-2.37 (m, 1H), 2.12-2.05 (m, 2H), 1.13-1.10 (m, 1H), 0.49-0.47 (m, 1H), 0.35-0.20 (m, 3H).

LC-MS: MS m/z (ESI): 386.0 $[M+H]^+$.

(*S*)-5-Cyclopropyl-5-(3-(isoindolin-2-yl)-3-oxopropyl)imidazolidine-2,4-dione (4)



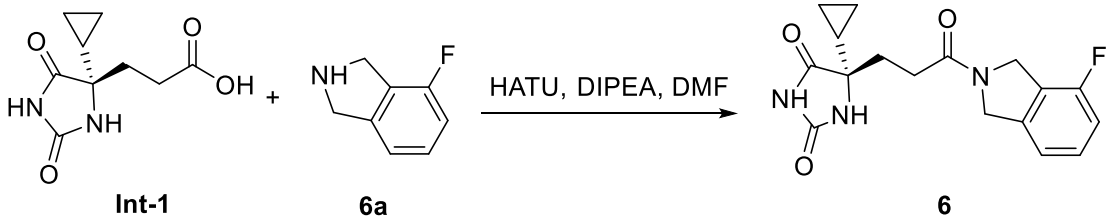
Int-1 4a 4

32 mg compound **4** was obtained from 55 mg **Int-1** and 31 mg **4a**, yield 39.4%.

^{1}H NMR (400 MHz, CD_3OD): δ 7.22-7.16 (m, 4H), 4.80-4.72 (m, 2H), 4.67-4.62 (m, 2H), 2.46-2.42 (m, 1H), 2.37-2.34 (m, 1H), 2.17-2.10 (m, 2H), 1.16-1.12 (m, 1H), 0.51-0.47 (m, 1H), 0.34-0.30 (m, 2H), 0.27-0.23 (m, 1H).

LC-MS: MS m/z (ESI): 314.2 $[M+H]^+$.

(*S*)-5-Cyclopropyl-5-(3-(4-fluoroisoindolin-2-yl)-3-oxopropyl)imidazolidine-2,4-dione (6)



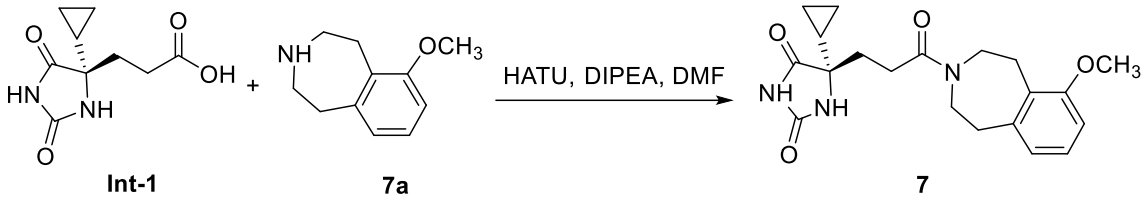
Int-1 6a 6

15 mg compound **6** was obtained from 53 mg **Int-1** and 34 mg **6a**, yield 18.1%.

^{1}H NMR (400 MHz, CD_3OD): δ 7.25-7.22 (m, 1H), 7.08-7.06 (m, 1H), 6.96-6.92 (m, 1H), 4.85-4.81 (m, 2H), 4.69-4.66 (m, 2 H), 2.47-2.42 (m, 1H), 2.36-2.34 (m, 1H), 2.12-2.09 (m, 2H), 1.17-1.14 (m, 1H), 0.50-00.47 (m, 1H), 0.33-0.31 (m, 2H), 0.27-0.23 (m, 1H).

LC-MS: MS m/z (ESI): 332.2 $[M+H]^+$.

(*S*)-5-Cyclopropyl-5-(3-(6-methoxy-1,2,4,5-tetrahydro-3*H*-benzo[d]azepin-3-yl)-3-oxopropyl)imidazolidine-2,4-dione (7)



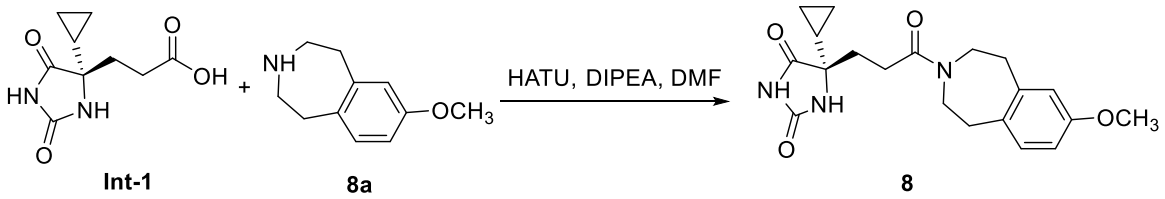
Int-1 7a 7

20 mg compound **7** was obtained from 34 mg **Int-1** and 28 mg **7a**, yield 33.9%.

^{1}H NMR (400 MHz, CD_3OD): δ 7.13-7.09 (m, 1H), 6.86-6.78 (m, 1H), 6.75-6.66 (m, 1H), 3.82 (s, 3H), 3.72-3.60 (m, 4H), 3.16-2.68 (m, 4H), 2.52-2.39 (m, 2H), 2.11-2.03 (m, 2H), 1.21-1.19 (m, 1H), 0.60-0.57 (m, 1H), 0.47-0.31 (m, 3H).

LC-MS: MS m/z (ESI): 372.2 $[M+H]^+$.

(*S*)-5-Cyclopropyl-5-(3-(7-methoxy-4,5-dihydro-1*H*-benzo[*d*]azepin-3(2*H*)-yl)-3-oxopropyl)imidazolidine-2,4-dione (8)



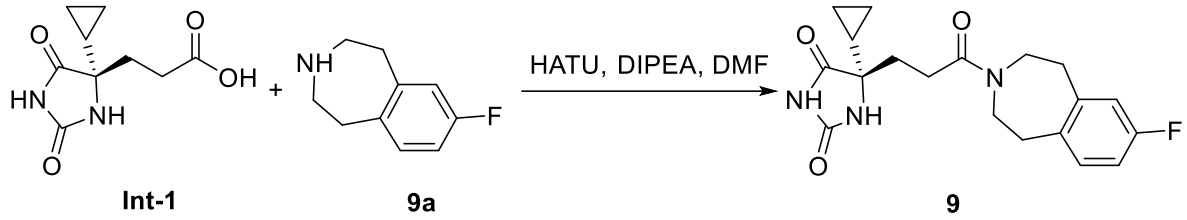
Int-1 8a 8

17 mg compound **8** was obtained from 39 mg **Int-1** and 32 mg **8a**, yield 25.1%.

^{1}H NMR (400 MHz, CD_3OD): 7.08-7.05 (m, 1H), 6.75-6.68 (m, 2H), 3.77 (s, 3H), 3.72-3.59 (m, 4H), 2.96-2.84 (m, 4H), 2.55-2.51 (m, 1H), 2.45-2.43 (m, 1H), 2.13-2.08 (m, 2H), 1.24-1.20 (m, 1H), 0.60-0.58 (m, 1H), 0.47-0.33 (m, 3H).

LCMS: MS m/z (ESI): 372.0 $[M+H]^+$.

(*S*)-5-Cyclopropyl-5-(3-(7-fluoro-1,2,4,5-tetrahydro-3*H*-benzo[d]azepin-3-yl)-3-oxopropyl)imidazolidine-2,4-dione (9)



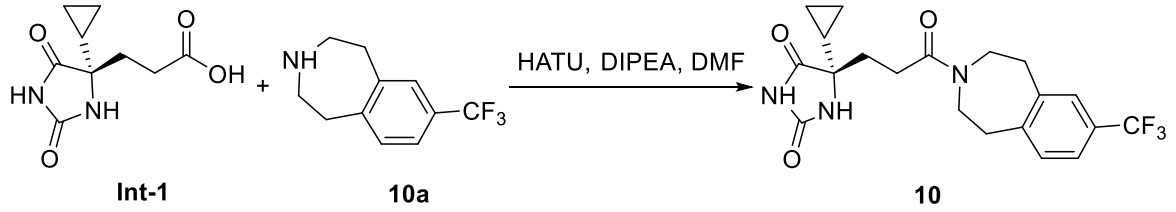
Int-1 9a 9

22 mg compound **9** was obtained from 41 mg **Int-1** and 32 mg **9a**, yield 31.6%.

^{1}H NMR (400 MHz, CD_3OD): δ 7.18-7.14 (m, 1H), 6.98-6.81 (m, 2H), 3.80-3.55 (m, 4H), 3.03-2.90 (m, 4H), 2.63-2.54 (m, 1H), 2.48-2.40 (m, 1H), 2.19-2.03 (m, 2H), 1.25-1.20 (m, 1H), 0.62-0.57 (m, 1H), 0.48-0.35 (m, 3H).

LCMS: MS m/z (ESI): 360.0 $[M+H]^+$.

(S)-5-Cyclopropyl-5-(3-oxo-3-(7-(trifluoromethyl)-1,2,4,5-tetrahydro-3H-benzo[d]azepin-3-yl)propyl)imidazolidine-2,4-dione (10)



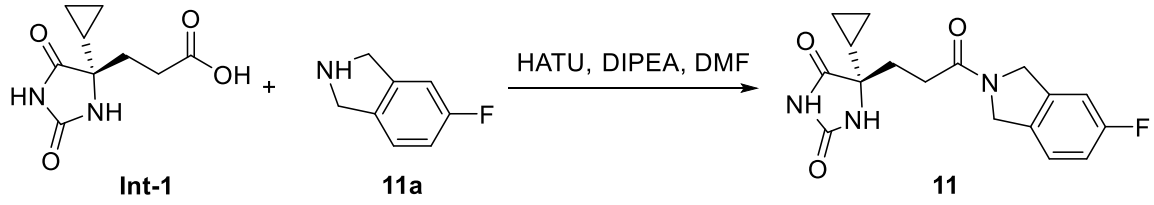
Int-1 10a 10

9 mg compound **10** was obtained from 27 mg **Int-1** and 27 mg **10a**, yield 17.3%.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.44 (s, 1H), 7.33-7.29 (m, 2H), 7.20-7.13 (m, 1H), 6.79 (brs, 1H), 3.69-3.49 (m, 4H), 2.92-2.81 (m, 4H), 2.4-2.44 (m, 2H), 2.23-2.14 (m, 2H), 1.14-1.10 (m, 1H), 0.49-0.44 (m, 1H), 0.37-0.28 (m, 3H).

LCMS: MS m/z (ESI): 410 $[M+H]^+$.

(*S*)-5-Cyclopropyl-5-(3-(5-fluoroisoindolin-2-yl)-3-oxopropyl)imidazolidine-2,4-dione (11)



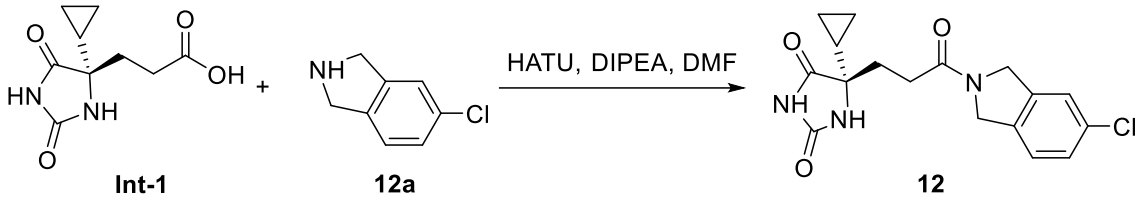
Int-1 11a 11

16 mg compound **11** was obtained from 24 mg **Int-1** and 16 mg **11a**, yield 42.1%.

^{1}H NMR (400 MHz, CD_3OD): δ 7.35-7.31 (m, 1H), 7.11-7.04 (m, 2H), 4.87 (d, *J* = 5.2 Hz, 2H), 4.72 (d, *J* = 6.0 Hz, 2H), 2.57-2.53 (m, 1H), 2.48-2.44 (m, 1H), 2.26-2.18 (m, 2H), 1.27-1.23 (m, 1H), 0.62-0.60 (m, 1H), 0.49-0.40 (m, 2H), 0.37-0.32 (m, 1H).

LC-MS: MS m/z (ESI): 332.2 $[M+H]^+$.

(*S*)-5-(3-(5-Chloroisoindolin-2-yl)-3-oxopropyl)-5-cyclopropylimidazolidine-2,4-dione (12)



Int-1 **12a** **12**

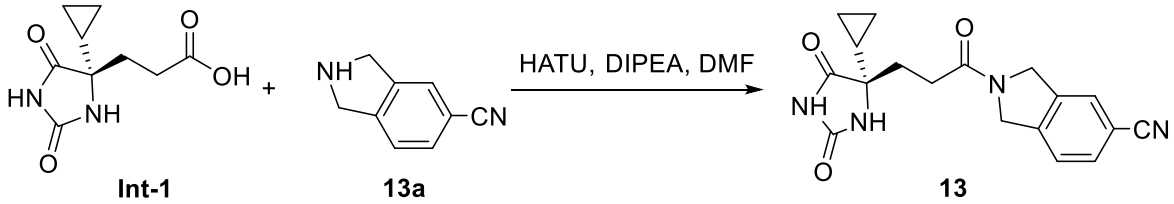
70 mg compound **12** was obtained from 134 mg **Int-1** and 100 mg **12a**, yield 38.0%.

^{1}HNMR (400 MHz, DMSO-*d6*): δ 10.62 (s, 1H), 7.73 (s, 1H), 7.45(s, 1H), 7.39-7.33 (m, 2H), 4.77(d, *J* = 6.8 Hz, 2H), 4.61 (d, *J* = 8.8 Hz, 2H), 2.43-2.33 (m, 1H), 2.31-2.22 (m, 1H), 2.00 (t, *J* = 8.0 Hz, 2H), 1.15-1.07 (m, 1H), 0.49-0.45 (m, 1H), 0.40-0.29 (m, 2H), 0.13-0.08 (m, 1H).

^{13}C NMR (400 MHz, DMSO-d_6) δ 178.0, 170.3, 157.3, 139.2, 135.8, 132.5, 127.9, 125.2, 123.6, 64.3, 52.0, 51.8, 31.9, 28.4, 16.6, 1.0, -0.5.

LCMS: MS m/z (ESI): 348.1 $[M+H]^+$.

(*S*)-2-(3-(4-Cyclopropyl-2,5-dioxoimidazolidin-4-yl)propanoyl)isoindoline-5-carbonitrile (13)



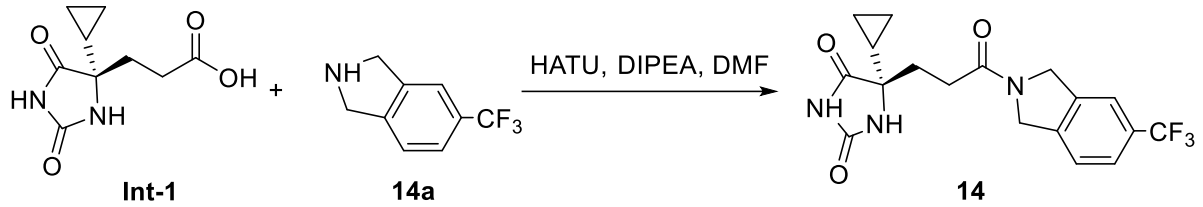
Int-1 **13a** **13**

25 mg compound **13** was obtained from 88 mg **Int-1** and 50 mg **13a**, yield 21.3%.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97-7.92 (m, 1H), 7.65-7.57 (m, 2H), 7.45-7.38 (m, 1H), 6.15 (brs, 1H), 4.84 (brs, 4H), 2.58-2.30 (m, 4H), 1.25-1.18 (m, 1H), 0.64-0.57 (m, 1H), 0.50-0.44 (m, 1H), 0.42-0.32 (m, 2H).

LCMS: MS m/z (ESI): 339.1 $[M+H]^+$.

(*S*)-5-Cyclopropyl-5-(3-oxo-3-(5-(trifluoromethyl)isoindolin-2-yl)propyl)imidazolidine-2,4-dione (14)



Int-1 **14a** **14**

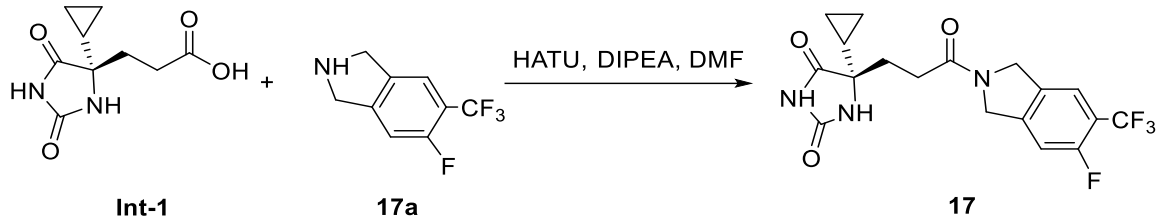
80 mg compound **14** was obtained from 170 mg **Int-1** and 150 mg **13a**, yield 26.2%.

^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.64 (s, 1H), 7.77-7.74 (m, 2H), 7.66 (d, *J* = 8.4 Hz, 1H), 7.58 (d, *J* = 8.0 Hz, 1H), 4.89-4.69 (m, 4H), 2.48-2.38 (m, 1H), 2.33-2.24 (m, 1H), 2.04-1.99 (m, 2H), 1.16-1.08 (m, 1H), 0.50-0.46 (m, 1H), 0.43-0.29 (m, 2H), 0.15-0.08 (m, 1H).

^{13}C NMR (400 MHz, DMSO-d_6) δ 178.0, 170.4, 157.3, 143.0, 142.5, 133.9, 133.3, 122.3, 112.4, 111.7, 64.3, 52.3, 51.2, 31.9, 28.4, 16.6, 1.0, -0.5.

LCMS: MS m/z (ESI): 382.1 $[M+H]^+$.

(*S*)-5-Cyclopropyl-5-(3-(5-fluoro-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)imidazolidine-2,4-dione (17)



Int-1 **17a** **17**

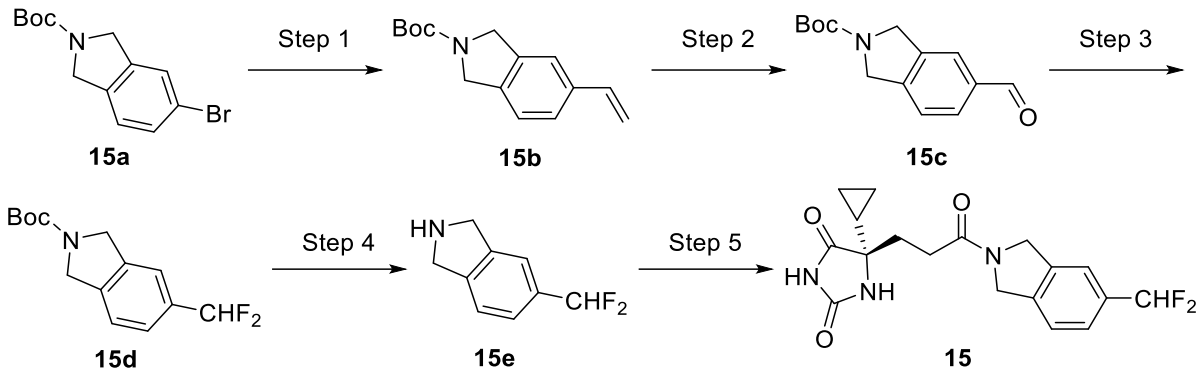
3.4 mg compound **17** was obtained from 5 mg **Int-1** and 5 mg **17a**, yield 37.1%.

^{1}H NMR (400 MHz, CD_3OD) δ 7.78-7.64 (m, 1H), 7.36 (t, *J* = 10.0 Hz, 1H), 4.91 (s, 1H), 4.82 (s, 2H), 4.78 (s, 1H), 2.59-2.53 (m, 1H), 2.49-2.41 (m, 1H), 2.29-2.19 (m, 2H), 1.28-1.23 (m, 1H), 0.63-0.58 (m, 1H), 0.49-0.33 (m, 3H).

^{19}F NMR (376.5 MHz, CD_3OD) δ -77.44, -62.82

LCMS: MS m/z (ESI): 400.0 $[M+H]^+$

(S)-5-Cyclopropyl-5-(3-(5-(difluoromethyl)isoindolin-2-yl)-3-oxopropyl)imidazolidine-2,4-dione (15)



Step 1: Tert-butyl 5-vinylisoindoline-2-carboxylate (15b)

To a mixture of **15a** (1 g, 3.35 mmol), K_2CO_3 (926 mg, 6.71 mmol) and potassium vinyltrifluoroborate (539 mg, 4.02 mmol) in 1,4-dioxane (10 mL) and water (2 mL) was added $Pd(dppf)Cl_2$ (197.54 mg, 335.37 μmol). The reaction was stirred under N_2 at 80 ℃ for 3 h. The reaction mixture was cooled to room temperature and diluted with water (50 mL), extracted with EtOAc (30 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography (hexane: EtOAc = 10: 1) to give **15b** (786 mg, yield 95.54%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-7.15 (m, 3H), 6.71 (dd, *J* = 16.4 Hz, 10.8 Hz, 1H), 5.73 (dd, *J* = 17.6 Hz, 2.8 Hz, 1H), 5.23 (d, *J* = 10.8 Hz, 1H), 4.67 (br, 2H), 5.22 (br, *2*H), 1.52 (d, , *J* = 2.0 Hz, 9H).

Step 2: Tert-butyl 5-formylisoindoline-2-carboxylate (15c)

To a mixture of **15b** (400 mg, 1.63 mmol), $NaIO_4$ (698 mg, 3.26 mmol) in 1,4-dioxane (7 mL) and water (4 mL) was added osmium tetroxide (42 mg, 163.05 μmol). The reaction was stirred at room temperature for 0.5 h and then diluted with water (40 mL). The mixture was extracted with EtOAc (20 mL × 3), and the organic solution was washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography (hexane: EtOAc = 5:1) to give **15c** (98 mg, yield 24.30%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 10.01 (s, 1H), 7.82-7.76 (m, 2H), 7.44-7.40 (m, 1H), 4.76-7.73 (m, 4H), 1.53 (s, 9H).

Step 3: Tert-butyl 5-(difluoromethyl)isoindoline-2-carboxylate (15d)

To a mixture of **15c** (50 mg, 202.19 μmol) and EtOH (0.93 mg, 20.22 μmol) in DCM (3 mL) was added DAST (162.96 mg, 1.01 mmol). The reaction was stirred at room temperature for 16 h. The reaction was quenched with water (30 mL), and the mixture was extracted with EtOAc

(20 mL × 3). The organic solution was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel chromatography (hexane: EtOAc = 4:1) to give title compound **15d** (52 mg, yield 95.50%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36-7.19 (m, 3H), 6.57 (t, *J* = 56.4 Hz, 1H), 4.65 (br, *2*H), 4.61 (br, 2H), 1.45 (s, 9H).

^{19}F NMR (400 MHz, $CDCl_3$): δ -109.86.

Step 4: 5-(Difluoromethyl)isoindoline (15e)

To the mixture of **15d** (30 mg, 111.41 μmol) in DCM (3 mL) was add HCl/dioxane (1N, 1 mL, 1 mmol). The reaction was stirred at room temperature for 16 h. The mixture was concentrated to give the crude **15e** for next step as it is.

LCMS: MS m/z (ESI): 170.0 $[M+H]^+$.

Step 5: (*S*)-5-Cyclopropyl-5-(3-(5-(difluoromethyl)isoindolin-2-yl)-3-oxopropyl)imidazolidine-2,4-dione (15)

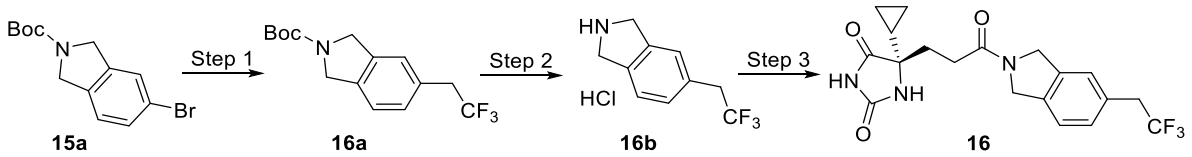
To a solution of crude **15e** (~ 111.4 μmol) in DMF (3 mL) was added **Int-1** (44 mg, 206.89 μmol), TEA (62.81 mg, 620.67 μmol) and HATU (94 mg, 248.27 μmol). The mixture was stirred at room temperature for 2 h. Water (30 mL) was added, and the mixture was extracted with EtOAc (20 mL × 2). The combined organic layer was washed with water (30 mL) and brine (30 mL), dried and concentrated. The crude product was purified by prep-HPLC to give **15** (5 mg, yield 6.65% over 2 steps).

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.64 (brs, 0.5H), 7.73 (brs, 0.5H), 7.73-7.70 (m, 1H), 7.58-7.49 (m, 2H), 7.05 (t, *J* = 56.0 Hz, 1H), 4.88-4.84 (m, 2H), 4.67-4.65 (m, 2H), 2.50-2.28 (m, 2H), 2.07-1.97 (m, 2H), 1.12-0.85 (m, 1H), 0.46-0.27 (m, 3H), 0.20-0.05 (m, 1H).

^{19}F NMR (376.5 MHz, DMSO-d_6): δ -108.63.

LCMS: MS m/z (ESI): 364.0 $[M+H]^+$.

(S)-5-Cyclopropyl-5-(3-oxo-3-(5-(2,2,2-trifluoroethyl)isoindolin-2-yl)propyl)imidazolidine-2,4-dione (16)



15a 16a 16b 16

Step 1: Tert-butyl 5-(2,2,2-trifluoroethyl)isoindoline-2-carboxylate (16a)

To a mixture of tert-butyl 5-bromoisoindoline-2-carboxylate **15a** (500 mg, 1.68 mmol) and 1,1,1-trifluoro-2-iodoethane (1.76 g, 8.38 mmol) in DMSO (3 mL) was added copper (1.07 g, 16.77 mmol). The reaction was stirred at 120 ℃ for 40 h. The reaction was cooled to room temperature, and water (100 mL) was added. The mixture was extracted with EtOAc (30 mL × 2), and the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The crude product was purified by prep-HPLC to give **16a** (33 mg, yield 6.53%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21-7.15 (m, 3H), 4.69-4.65 (m, 4H), 3.37 (q, *J* = 10.8 Hz, 2H), 1.52 (s, 9H).

^{19}FNMR (400 MHz, $CDCl_3$): δ -66.03.

Step 2: 5-(2,2,2-Trifluoroethyl)isoindoline hydrochloride (16b)

The mixture of **16a** (23 mg, 76.41 μmol) in DCM (3 mL) was add HCl/1,4-dioxane (1N, 1 mL, 1 mmol). The reaction was stirred at room temperature for 16 h. The mixture was concentrated to give crude **16b** which was used for next step directly.

LCMS: MS m/z (ESI): 202.2 $[M+H]^+$.

Step 3: (*S*)-5-Cyclopropyl-5-(3-(5-(difluoromethyl) isoindolin-2-yl)-3-oxopropyl) imidazolidine-2,4-dione (16)

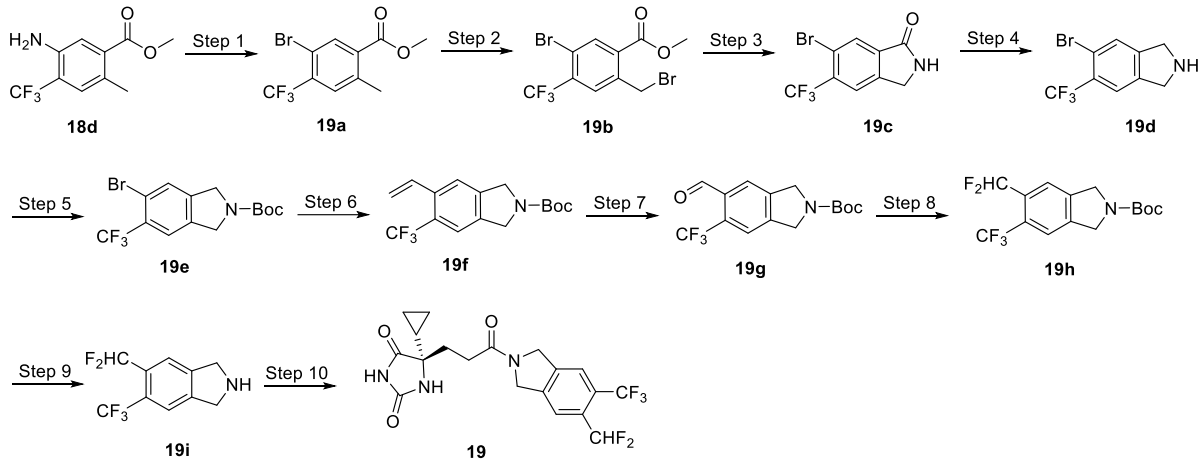
To a solution of **16b** (~76.41 μmol) in DMF (3 mL) was added **Int-1** (23 mg, 109.35 μmol), TEA (30 mg, 298.23 μmol) and HATU (45 mg, 119.29 μmol). The mixture was stirred at room temperature 2 h. Water (30 mL) was added, and the mixture was extracted with EtOAc (20 mL × 2). The combined organic layers were washed with water (30 mL) and brine (30 mL), dried and concentrated. The crude product was purified by prep-HPLC to give **16** (13 mg, yield 33.08%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 10.62 (brs, 1H), 7.74 (s, 1H), 7.37-7.27 (m, 3H), 4.80 (br, 2H), 4.62 (br, 2H), 3.67 (q, *J* = 11.6 Hz, 2H), 2.47-2.40 (m, 1H), 2.30-2.26 (m, 1H), 2.01 (t, *J* = 8.0 Hz, 2H), 1.13-1.09 (m, 1H), 0.47-0.31 (m, 3H), 0.13-0.10 (m, 1H).

^{19}F NMR (400 MHz, $CDCl_3$): δ -64.44.

LCMS: MS m/z (ESI): 396.1 $[M+H]^+$.

(S)-5-Cyclopropyl-5-(3-(5-(difluoromethyl)-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)imidazolidine-2,4-dione (19)



Step 1: Methyl 5-bromo-2-methyl-4-(trifluoromethyl)benzoate (19a)

To a suspension of **18d** (500 mg, 2.14 mmol) in CH_3CN (20 mL) was added isoamyl nitrite (377 mg, 3.22 mmol) and $CuBr_2$ (960 mg, 4.30 mmol). After the mixture was stirred at 70 ℃ overnight, the mixture was cooled down to room temperature and poured into ice water (20 mL). Then the mixture was extracted with EtOAc (50 mL). The organic phase was dried over Na_2SO_4 and the solid was filtered off. The filtrate was concentrated to afford crude **19a** (600 mg, yield 94.20%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (s, 1H), 7.56 (s, 1H), 3.93 (s, 3H), 2.59 (s, 3H).

Step 2: Methyl 5-bromo-2-(bromomethyl)-4-(trifluoromethyl)benzoate (19b)

To a solution of **19a** (100 mg, 336.62 μmol) in CCl_4 (3 mL) was added AIBN (2 mg, 10.10 μmol) and NBS (72 mg, 403.95 μmol). The mixture was stirred at 70 ℃ overnight. The mixture was cooled to room temperature and filtered. The cake was washed with DCM and the filtrate was concentrated in vacuo to give crude **19b** (150 mg, yield 118.52%) as a yellow oil for next step as it is.

Step 3: 6-Bromo-5-(trifluoromethyl)isoindolin-1-one (19c)

To a solution of **19b** (150 mg, 398.97 μmol) in MeOH (1 mL) was added NH_3/MeOH (4 mL), the mixture was stirred at room temperature overnight. The reaction mixture was concentrated in vacuo, and the residue was purified by silica gel chromatography (EtOAc/hexane=1/5) to give **19c** (60 mg, yield 53.70% over 2 steps) as a white solid.

^{1}HNMR (400 MHz, DMSO-*d6*): δ 9.03 (brs, 1H), 8.16 (s, 1H), 8.08 (s, 1H), 4.44 (s, 2H).

LCMS: MS m/z (ESI): 280.3 $[M+H]^+$.

Step 4: 5-Bromo-6-(trifluoromethyl)isoindoline (19d)

To a solution of **19c** (60 mg, 214.25 μmol) in THF (2 mL) was added BH_3/THF (1.0 M, 2.1 mL, 2.1 mmol). The mixture was heated to 60 ℃ and stirred overnight. The reaction was quenched with MeOH (5 mL), and the mixture was adjusted pH = 1-2 with 6M HCl. The mixture was heated to 80 ℃ and stirred for 1h. The reaction was cooled to room temperature and adjusted pH to 7-8 with 6 M NaOH. The mixture was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (MeOH/DCM=1/20) to afford **19d** (20 mg, yield 35.09%) as a purple solid.

LCMS: MS m/z (ESI): 266.2 $[M+H]^+$.

Step 5: Tert-butyl 5-bromo-6-(trifluoromethyl)isoindoline-2-carboxylate (19e)

To a solution of **19d** (700 mg, 2.63 mmol) in THF (15 mL) was added TEA (1.5 mL) and $(Boc)_2O$ (689 mg, 3.16 mmol), and the mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated in vacuum and the residue was purified by silica gel column chromatography (ethyl acetate/hexane =1/20) to afford **19e** (750 mg, yield 77.85%) as a white solid.

LCMS: MS m/z (ESI): 310.9 $[M+H\text{-}tBu]^+$.

Step 6: Tert-butyl 5-(trifluoromethyl)-6-vinylisoindoline-2-carboxylate (19f)

To a solution of **19e** (750 mg, 2.05 mmol) in 1,4-dioxane (30 mL) was added potassium vinyltrifluoroborate (302 mg, 2.25 mmol), $Pd(dppf)Cl_2$ (167 mg, 204.82 μmol) and K_2CO_3 (849 mg, 6.14 mmol). The reaction was replaced with N_2 for three times. The mixture was stirred at 90 ℃ overnight. Water was added and the mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The residue was purified by prep-HPLC to give **19f** (500 mg, yield 77.91%) as a white solid.

^{1}HNMR (400 MHz, DMSO-*d6*): δ7.81 (d, *J* = 6.8 Hz, 1H), 7.71 (d, *J* = 5.2 Hz, 1H), 7.02-6.94 (m, 1H), 5.94-5.87 (m, 1H), 5.50 (d, *J* = 12.0 Hz, 1H), 4.66-4.58 (m, 4H), 1.47 (s, 9H).

LCMS: MS m/z (ESI): 258.4 $[M+H\text{-}tBu]^+$.

Step 7: Tert-butyl 5-formyl-6-(trifluoromethyl)isoindoline-2-carboxylate (19g)

To a solution of **19f** (200 mg, 638.34 μmol) in 1,4-dioxane (2 mL) was added $NaIO_4$ (273 mg, 1.28 mmol) and H_2O (1 mL), and the mixture was stirred at room temperature. Then OsO_4 (16 mg, 63.83 μmol) was added, and the reaction mixture was stirred at room temperature for 3 h. Saturated sodium bicarbonate was added, and the reaction mixture was extracted with EtOAc.

The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The residue was purified by silica gel chromatography (EtOAc: hexane = 1: 20) to give **19g** (170 mg, yield 84.47%).

LCMS: MS m/z (ESI): 260.4 $[M+H\text{-}tBu]^+$.

Step 8: Tert-butyl 5-(difluoromethyl)-6-(trifluoromethyl)isoindoline-2-carboxylate (19h)

To a solution of **19g** (170 mg, 539.19 μmol) in DCM (5 mL) was added EtOH (2.5 mg, 53.92 μmol). DAST (435 mg, 2.70 mmol) was added dropwise at room temperature. The reaction was stirred at room temperature for 3 h. Water was added, and the reaction mixture was extracted with DCM. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The residue was purified by silica gel chromatography eluting with ethyl acetate/hexane (1:20) to afford **19h** (170 mg, yield 93.48%).

LCMS: MS m/z (ESI): 338.3 $[M+H]^+$.

Step 9: 5-(Difluoromethyl)-6-(trifluoromethyl)isoindoline (19i)

The solution of **19h** (170 mg, 504.03 μmol) in HCl/1,4-dioxane (4N, 5 mL, 20 mmol) was stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuum to give **19i** (85 mg, yield 71.11%).

LCMS: MS m/z (ESI): 238.1 $[M+H]^+$.

Step 10: (S)-5-Cyclopropyl-5-(3-(5-(difluoromethyl)-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)imidazolidine-2,4-dione (19)

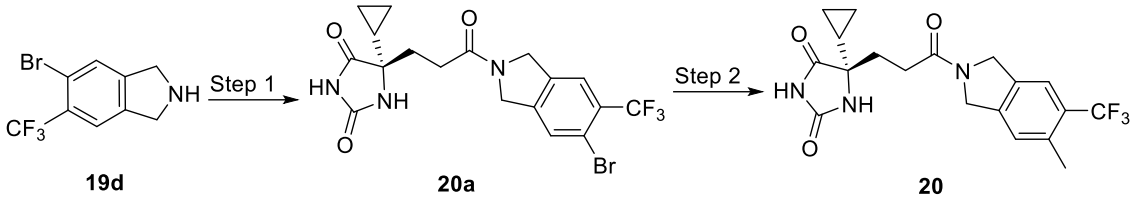
To a solution of **19i** (40 mg, 168.66 μmol) in DMF (2 mL) was successively added TEA (0.2 mL), **Int-1** (38 mg, 179.07 μmol) and HATU (70 mg, 184.10 μmol). The mixture was stirred at room temperature for 2 h. Water was added, and the reaction mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The crude mixture was purified by prep-HPLC to give **19** (5 mg, yield 6.87%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.64 (s, 1H), 7.95-7.93 (m, 2H), 7.76 (s, 1H), 7.28 (t, 1H), 4.93-4.91 (m, 2H), 4.75-4.73 (m, 2H), 2.46-2.40 (m, 1H), 2.32-2.23 (m, 1H), 2.06-1.97 (m, 2H), 1.16-1.08 (m, 1H), 0.50-0.29 (m, 3H), 0.15-0.08 (m, 1H).

^{19}F NMR (376.5 MHz, DMSO-*d6*) δ -56.72, -109.89.

LCMS: MS m/z (ESI): 432.4 $[M+H]^+$.

(S)-5-Cyclopropyl-5-(3-(5-methyl-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)imidazolidine-2,4-dione (20)



19d 20a 20

Step 1: (*S*)-5-(3-(5-Bromo-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)-5-cyclopropylimidazolidine-2,4-dione (20a)

To a solution of **19d** (20 mg, 75.17 μmol) in DMF (1.5 mL) was added TEA (0.2 mL), **Int-1** (16 mg, 75.40 μmol) and HATU (30 mg, 78.90 μmol). The mixture was stirred at room temperature for 2 h. Water was added, and the mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by prep-HPLC to give **20a** (10 mg, yield 28.90%).

LCMS: MS m/z (ESI): 460.3 $[M+H]^+$.

Step 2: (S)-5-Cyclopropyl-5-(3-(5-methyl-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)imidazolidine-2,4-dione (20)

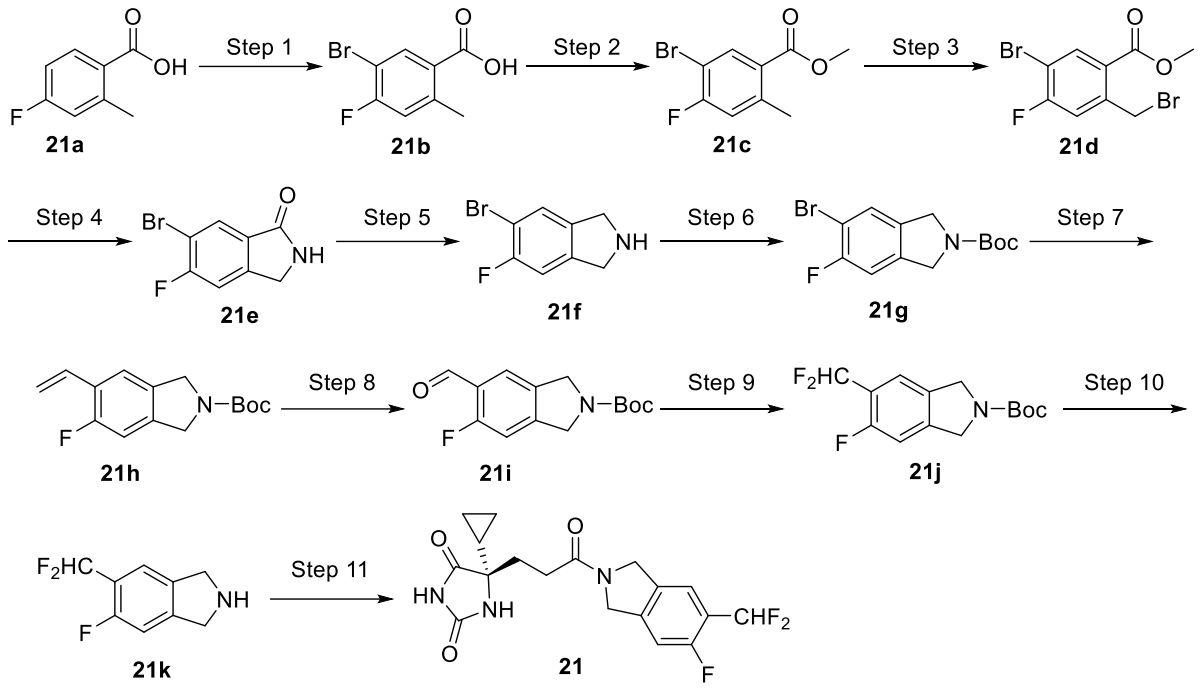
To a solution of **20a** (80 mg, 173.82 μmol) in 1,4-dioxane (5 mL) was added water (5 mL), Cs_2CO_3 (230 mg, 705.91 μmol), $Pd(dppf)Cl_2$ (13 mg, 17.38 μmol) and methylboronic acid (21 mg, 347.64 μmol). The mixture was protected with N_2 and stirred at 90 ℃ overnight. Water was added and the mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by prep-HPLC to give **20** (5 mg, yield 7.28%) as white solid.

^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.62 (s, 1H), 8.36 (s, 1H), 7.75 (s, 1H), 7.69 (s, 1H), 7.42 (d, *J* = 3.6 Hz, 1H), 4.82 (d, *J* = 6.4 Hz, 2H), 4.64 (d, *J* = 3.2 Hz, 2H), 2.44 (s, 3H), 2.33-2.22 (m, 2H), 2.01 (t, *J* = 8.0 Hz, 2H), 1.14-1.08 (s, 1H), 0.50-0.31 (m, 3H), 0.15-0.08 (m, 1H).

^{19}F NMR (376.5 MHz, DMSO-*d6*): δ -59.92.

LCMS: MS m/z (ESI): 396.4 $[M+H]^+$.

(S)-5-Cyclopropyl-5-(3-(5-(difluoromethyl)-6-fluoroisoindolin-2-yl)-3-oxopropyl)imidazolidine-2,4-dione (21)



Step 1: 5-Bromo-4-fluoro-2-methylbenzoic acid (21b)

To a solution of 4-fluoro-2-methylbenzoic acid **21a** (10 g, 64.88 mmol) in H_2SO_4 (50 mL) was added NBS (11.6 g, 65.18 mmol) in portions at 0 ℃. The reaction mixture was stirred at 0-5 ℃ for 2 h. The resulting mixture was poured into ice-water. The solid was collected by filtration and dried in vacuum to give **21b** (14 g, yield 92.60%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.07 (d, J = 3.6 Hz, 1H), 7.39 (d, J = 5.0 Hz, 1H), 2.51 (s, 3H).

Step 2: Methyl 5-bromo-4-fluoro-2-methylbenzoate (21c)

To a solution of **21b** (15 g, 64.37 mmol) in MeOH (150 mL) at 0 ℃ was added $SOCl_2$ (22.97 g, 193.10 mmol, 14 mL) slowly. The reaction mixture was heated to 80 ℃ for 2 h. Then the mixture was cooled to room temperature and concentrated in vacuum. The residue was diluted with aqueous NH_4Cl and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuum to afford crude **21c** (15.2 g, yield 95.58%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.02 (d, J = 3.6 Hz, 1H), 7.37 (d, J = 5.0 Hz, 1H), 3.78 (s, 3H), 2.45 (s, 3H).

Step 3: Methyl 5-bromo-2-(bromomethyl)-4-fluorobenzoate (21d)

To a solution of **21c** (15.2 g, 61.52 mmol) in CCl_4 (250 mL) was added NBS (13.14 g, 73.83 mmol) and AIBN (1.01 g, 6.15 mmol). The reaction mixture was stirred at 80 ℃ overnight. Then the mixture was cooled down to room temperature and filtered. The cake was washed with CCl_4, the filtrate was concentrated in vacuum. The residue was purified by silica gel chromatography (EtOAc: hexane=1:20) to afford **21d** (19 g, yield 94.74%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.16 (d, J = 3.6 Hz, 1H), 7.71 (d, J = 5.0 Hz, 1H), 4.98 (s, 2H), 3.88 (s, 3H).

Step 4: 6-Bromo-5-fluoroisoindolin-1-one (21e)

The solution of **21d** (4.6 g, 14.11 mmol) in NH_3/MeOH (7N, 40 mL, 280 mmol) was stirred at room temperature overnight. The reaction mixture was concentrated in vacuum, the residue was purified by silica gel chromatography (MeOH: DCM=1:50) to give **21e** (3.0 g, yield 92.41%).

LCMS: MS m/z (ESI): 230.3 $[M+H]^+$.

Step 5: 5-Bromo-6-fluoroisoindoline (21f)

To a solution of **21e** (3.0 g, 13.04 mmol) in THF (20 mL) was added BH_3/THF (1N in THF, 90 mL, 90 mmol) and the mixture was heated to 65 ℃ overnight. The reaction was quenched with methanol (5 mL) and 6M HCl to adjust pH = 2. The mixture was heated to 80 ℃ for 2 h and cooled to room temperature. The mixture was adjusted to pH = 7-8 with 6M NaOH and extracted with ethyl acetate (3 X 100 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuum. The crude mixture was purified by silica gel chromatography (MeOH: DCM=1:20) to afford **21f** (350 mg, yield 12.42%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.62 (d, J = 3.2 Hz, 1H), 7.33 (d, J = 4.4 Hz, 1H), 4.14-4.10 (m, 4H).

LCMS: MS m/z (ESI): 216.2 $[M+H]^+$.

Step 6: Tert-butyl 5-bromo-6-fluoroisoindoline-2-carboxylate (21g)

To a solution of **21f** (350 mg, 1.62 mmol) in DCM (5 mL) was added Et_3N (492 mg, 4.86 mmol) and Boc_2O (425 mg, 1.94 mmol), the mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated in vacuum. The crude mixture was purified by silica gel chromatography (EtOAc: hexane=1:20) to afford **21g** (580 mg, yield 113.24%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.71-7.67 (m, 1H), 7.40-7.35 (m, 1H), 4.55-4.52 (m, 4H), 1.47-1.44 (m, 9H).

LCMS: MS m/z (ESI): 316.2 $[M+H]^+$.

Step 7: Tert-butyl 5-fluoro-6-vinylisoindoline-2-carboxylate (21h)

To a solution of **21g** (580 mg, 1.83 mmol) in 1,4-dioxane (20 mL) and H_2O (3 mL) was added potassium trifluoro(vinyl)borate (270 mg, 2.02 mmol), $Pd(dppf)Cl_2$ (150 mg, 183.45 μmol) and K_2CO_3 (760 mg, 5.50 mmol). The reaction was refilled with N_2 for three times. The mixture was stirred at 100 ℃ overnight. Water was added and the reaction mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The crude mixture was purified by silica gel chromatography (EtOAc: hexane=1: 20) to afford **21h** (400 mg, yield 82.81%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.63-7.58 (m, 1H), 7.22-7.17 (m, 1H), 6.86-6.79 (m, 1H), 5.89 (dd, J = 8.8 Hz, 1H), 5.41 (d, J = 6.0 Hz, 1H), 4.58-4.52 (m, 4H), 1.45 (s, 9H).

LCMS: MS m/z (ESI): 208.0 $[M+H\text{-}tBu]^+$.

Step 8: Tert-butyl 5-fluoro-6-formylisoindoline-2-carboxylate (21i)

To solution of **21h** (400 mg, 1.52 mmol) in 1,4-dioxane (8 mL) was added $NaIO_4$ (650 mg, 3.04 mmol) and H_2O (2 mL), and the mixture was stirred at room temperature. Then OsO_4 (39 mg, 151.91 μmol) was added. The reaction mixture was stirred at room temperature for 3 h. Saturated sodium bicarbonate was added, and then the reaction mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The crude mixture was purified by silica gel chromatography (EtOAc:hexane=1:20) to give **21i** (180 mg, yield 44.67%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.20 (s, 1H), 7.81-7.76 (m, 1H), 7.44-7.39 (m, 1H), 4.67-4.58 (m, 4H), 1.45 (s, 9H).

LCMS: MS m/z (ESI): 210.4 $[M+H\text{-}tBu]^+$.

Step 9: Tert-butyl 5-(difluoromethyl)-6-fluoroisoindoline-2-carboxylate (21j)

To a solution of **21i** (180 mg, 678.53 μmol) in DCM (5 mL) was added EtOH (3.1 mg, 67.85 μmol), then DAST (547 mg, 3.39 mmol) was added dropwise at room temperature. The mixture was stirred at room temperature for 3 h. Water was added, and the reaction mixture was extracted with DCM. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The crude mixture was purified by silica gel chromatography (EtOAc: hexane=1:20) to afford **21j** (180 mg, yield 92.34%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.62-7.58 (m, 1H), 7.40-7.33 (m, 1H), 7.20 (t, J = 46.4 Hz, 1H), 4.64-4.57 (m, 4H), 1.45 (s, 9H).

LCMS: MS m/z (ESI): 232.4 $[M+H\text{-}tBu]^+$.

Step 10: 5-(Difluoromethyl)-6-fluoroisoindoline (21k)

To a flask with **21j** (180 mg, 626.57 µmol) was added HCl/1,4-dioxane (4N, 10 mL, 10 mmol). The mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuum to give **21k** (110 mg, yield 93.80%).

LCMS: MS m/z (ESI): 188.1 $[M+H]^+$.

Step 11: (*S*)-5-Cyclopropyl-5-(3-(5-(difluoromethyl)-6-fluoroisoindolin-2-yl)-3-oxopropyl)imidazolidine-2,4-dione (21)

To a solution of **21k** (110 mg, 587.73 µmol) in DMF (5 mL) was added TEA (0.4 mL), **Int-1** (125 mg, 587.73 µmol) and HATU (246 mg, 646.50 µmol). The mixture was stirred at room temperature for 2 h. Water was added and reaction mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The residue was purified by prep-HPLC to give **21** (24 mg, yield 10.71%).

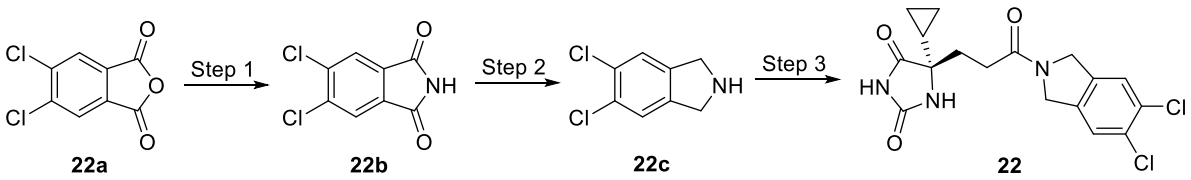
^{1}H NMR (400 MHz, DMSO-d_6): δ 10.51 (brs, 1H), 7.62 (s, 1H), 7.51 (d, J = 3.2 Hz, 1H), 7.28 (dd, J = 5.4 Hz, 1H), 7.09 (t, J = 54.4 Hz, 1H), 4.73-4.68 (m, 2H), 4.55-4.50 (m, 2H), 2.33-2.25 (m, 1H), 2.20-2.10 (m, 1H), 1.91-1.86 (m, 2H), 1.08-0.96 (m, 1H), 0.38-0.17 (m, 3H), 0.03-0.00 (m, 1H).

^{13}C NMR (400 MHz, DMSO-d_6) δ 178.0, 171.0, 157.3, 143.0, 142.4, 133.9, 133.3, 122.4, 112.4, 111.7, 64.3, 52.3, 51.6, 31.9, 28.4, 16.6, 1.0, -0.5.

^{19}F NMR (376.5 MHz, DMSO-d_6): δ -112.85, -120.80.

LCMS: MS m/z (ESI): 382.4 $[M+H]^+$.

(S)-5-Cyclopropyl-5-(3-(5,6-dichloroisoindolin-2-yl)-3-oxopropyl)imidazolidine-2,4-dione (22)



Step 1: 5,6-Dichloroisoindoline-1,3-dione (22b)

The mixture of **22a** (5 g, 23.04 mmol) in formamide (1.04 g, 23.04 mmol) was stirred at 200 ℃ for 2 h. The mixture was poured into ice water (100 mL), the mixture was filtered and the solid was dried to afford the tittle compound **22b** (4.5 g, yield 90.41%) as a brown solid.

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.62 (s, 1H), 8.12 (s, 2H).

Step 2: 5,6-Dichloroisoindoline (22c)

To a solution of **22b** (1 g, 4.63 mmol) in THF (5 mL) was added BH_3 (1 M in THF, 46.29 mL, 46.29 mmol). The resulting mixture was stirred at 80 ℃ for 16 h. Concentrated HCl (10 mL) was added to quench the reaction. Then aqueous NaOH was added to adjust pH~13 and the aqueous phase was extracted with EtOAc (80 mL X 3). The combined organic phase was washed with brine (80 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (DCM/MeOH=10/1) to afford the tittle compound **22c** (150 mg, yield 17.23%) as a brown solid.

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.55 (s, 2H), 4.06 (s, 4H).

Step 3: (S)-5-Cyclopropyl-5-(3-(5,6-dichloroisoindolin-2-yl)-3-oxopropyl)imidazolidine-2,4-dione (22)

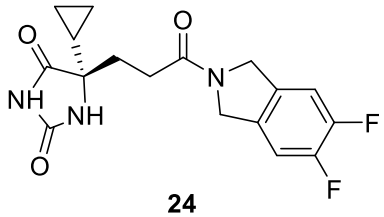
To a solution of **Int-1** (140 mg, 659.75 μmol) in DMF (7 mL) was added DIEA (111 mg, 857.67 μmol). The resulting mixture was followed by the addition of **22c** (149 mg, 791.70 μmol) and EDCI (116 mg, 857.67 μmol). The resulting mixture was stirred at room temperature for 2 h. The mixture was purified by prep-HPLC to afford the tittle compound **22** (109 mg, yield 43.0%) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.63 (s, 1H), 7.74 (s, 1H), 7.67 (s, 2H), 4.77 (brs, 2H), 4.60 (brs, 2H), 2.50-2.37 (m, 1H), 2.29-2.24 (m, 1H), 2.02-1.97 (m, 2H), 1.12-1.10 (m, 1H), 0.46-0.33 (m, 3H), 0.13-0.11 (m, 1H).

^{13}C NMR (400 MHz, DMSO-d_6) δ 178.0, 170.3, 157.3, 138.6, 138.0, 130.5, 130.3, 125.7, 125.6, 64.3, 51.8, 31.9, 28.4, 16.6, 1.0, -0.5.

LCMS: MS m/z (ESI): 382.0 $[M+H]^+$.

(*S*)-5-Cyclopropyl-5-(3-(5,6-difluoroisoindolin-2-yl)-3-oxopropyl)imidazolidine-2,4-dione (24)



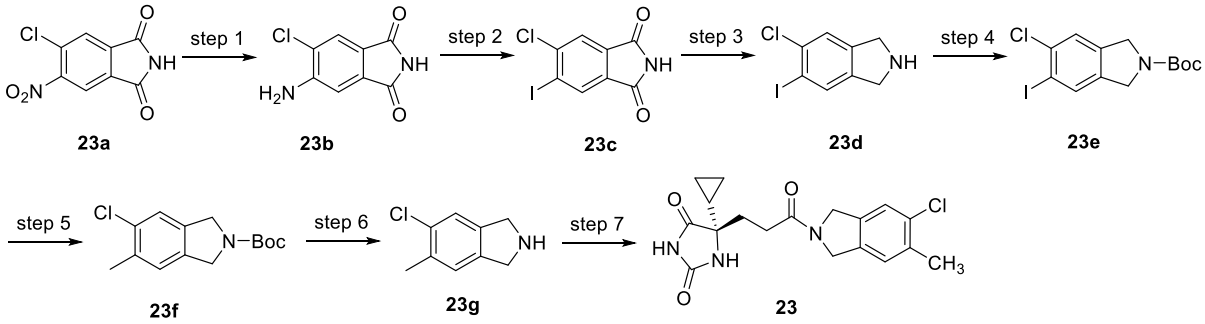
24

Compound **24** was prepared using 5,6-difluoroisobenzofuran-1,3-dione as starting material by following the same procedure of **22**.

^{1}H NMR (400 MHz, CD_3OD) δ 7.27 (dt, *J* = 10.1, 7.0 Hz, 2H), 4.85 (s, 2H), 4.72 (s, 2H), 2.58-2.53 (m, 1H), 2.45-2.41 (m, 1H), 2.29-2.18 (m, 2H), 1.32-1.18 (m, 1H), 0.64-0.58 (m, 1H), 0.52- 0.23 (m, 3H).

LC-MS: MS m/z (ESI): 350.2 $[M+H]^+$.

(*S*)-5-(3-(5-Chloro-6-methylisoindolin-2-yl)-3-oxopropyl)-5-cyclopropylimidazolidine-2,4-dione (23)



Step 1: 5-Amino-6-chloroisoindoline-1,3-dione (23b)

To a mixture of 5-chloro-6-nitroisoindoline-1,3-dione **23a** (1 g, 4.4 mmol) in MeOH (20 mL) was added ammonia solution (7 N in methanol, 3 mL, 21 mmol), H_2O (5 mL) and $Na_2S_2O_4$ (7.6 g, 44 mmol). The reaction was stirred at room temperature for 24 h. Water (4 mL) was added, and the mixture was extracted with EtOAc (30 mL X 4). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated to afford **23b** (0.5 g, yield 56%). The residue was used in next step without further purification.

LCMS: m/z (ESI): 197.2 $[M+H]^+$.

Step 2: 5-Chloro-6-iodoisoindoline-1,3-dione (23c)

To a stirred suspension of **23b** (0.5 g 2.5 mmol) in H_2O (15 mL), a solution of concentrated H_2SO_4 (0.4 mL) in H_2O (5 mL) was added dropwise at 10 °C. After the mixture was cooled to

5 ℃, a solution of sodium nitrite (276 mg, 4 mmol) in H_2O (5 mL) was added dropwise and the stirring continued at 0 ℃ for 90 min. Then a solution of potassium iodide (1.4 g, 8.8 mmol) in H_2O (8 mL) was added dropwise over 40 min while maintaining the reaction temperature between 0-5 ℃. The reaction mixture was warmed to room temperature and subsequently heated at 35 ℃ for 45 min and then 60 ℃ for 30 min. Then the mixture was cooled down to room temperature and was extracted with EtOAc (30 mL X 4). The combined organic layers were dried, filtered, and concentrated. The residue was resuspended in 30 mL DCM, stirred for 10 min at room temperature and the resulting crystals collected by filtration to yield compound **23c** (240 mg, yield 31%).

LCMS: m/z (ESI): 307.9 $[M+H]^+$.

Step 3: 5-Chloro-6-iodoisoindoline (23d)

To a solution of **23c** (120mg, 0.38 mmol) in THF (5 mL) was added borane–tetrahydrofuran (1M, 60 mL, 60 mmol) dropwise under N_2. The resulting mixture was stirred at 60 ℃ for 24 h. The reaction mixture was cooled to ambient temperature and quenched with MeOH (6 mL). Then 4N aqueous HCl (2 mL) was added, and the mixture was heated at 80 ℃ for 3 h. The mixture was cooled down to room temperature and 5N KOH was added to adjust pH = 7. The mixture was concentrated under reduced pressure and the residue was purified by silica gel chromatography (DCM: MeOH = 10: 1 with 2% NH_4OH as additive) to afford **23d** (51 mg, yield 47%).

LCMS: m/z (ESI): 280.0 $[M+H]^+$.

Step 4: Tert-butyl 5-chloro-6-iodoisoindoline-2-carboxylate (23e)

To a mixture of **23d** (40 mg, 0.143 mmol) in 5N KOH solution (2 mL) was added di-tert-butyl dicarbonate (100 mg, 0.45 mmol). The reaction was stirred at room temperature for 18 h. The mixture was cooled to 0 ℃, then filtered to give **23e** (28 mg, yield 63%).

LCMS: m/z (ESI): 380.0 $[M+H]^+$.

Step 5: Tert-butyl 5-chloro-6-methylisoindoline-2-carboxylate (23f)

To a solution of **23e** (20 mg, 0.052 mmol) in DME (3 mL) was added H_2O (2 mL), $CH_3B(OH)_2$ (20 mg 0.33 mmol), K_2CO_3 (15 mg 0.1 mmol) and $Pd(PPh_3)_2Cl_2$ (3 mg, 0.0052 mmol). The reaction mixture was stirred at 80 ℃ for 18 h under N_2 atmosphere. After the reaction was completed, the reaction mixture was quenched and extracted with EtOAc (20 mL X

2). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated to afford crude **23f**, which was used for next step without purification.

LCMS: m/z (ESI): 268.2 $[M+H]^+$.

Step 6: 5-Chloro-6-methylisoindoline (23g)

To a solution of **23f** (20 mg, 0.052 mmol) in DCM (3 mL) was added HCl in 1,4-dioxane (4N, 2 mL, 8 mmol). After the reaction was complete, the mixture was concentrated under reduced pressure and the residue was purified by silica gel chromatography (DCM: MeOH= 10: 1 with 2% NH_4OH as additive) to afford **23g** (5 mg, yield 60%).

LCMS: m/z (ESI): 168.2 $[M+H]^+$.

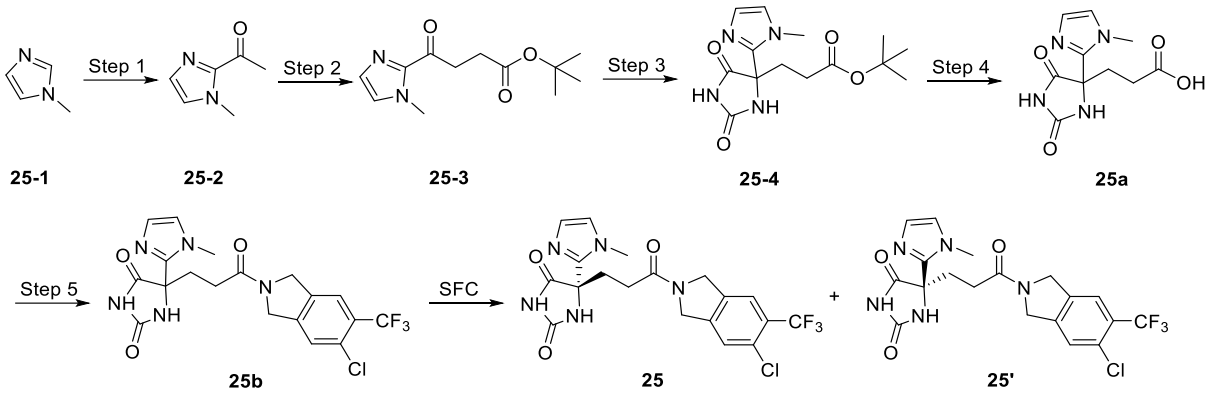
Step 7: (*S*)-5-(3-(5-Chloro-6-methylisoindolin-2-yl)-3-oxopropyl)-5-cyclopropylimidazolidine-2,4-dione (23)

To a mixture of **23g** (5 mg, 0.029 mmol) in DMF (2 mL) was added triethylamine (13 mg, 0.1mmol), **Int-1** (5 mg, 0.022 mmol) and HATU (12 mg, 0.031 mmol). The reaction was stirred at room temperature for 18 h. H_2O (3 mL) was added, and the mixture was extracted with EtOAc (20 mL X 2). The combined layer was washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified with prep-HPLC to give title compound **23** (3 mg, yield 37.7 %).

^{1}H NMR (400 MHz, Methanol-d_4): δ 7.36 (d, J = 4.0 Hz, 1H), 7.28 (d, J = 4.0 Hz, 1H), 4.83 (d, d, J = 4.0 Hz, 2H), 4.71 (d, J = 4.0 Hz, 2H), 2.56-2.54 (m, 1H), 2.52-2.43 (m, 1H) 2.39 (s, 3H), 2.26-2.20 (m, 2H), 1.27-1.23 (m, 1H), 0.62-0.60 (m, 1H), 0.48-0.40 (m, 2H), 0.36-0.34 (m, 1H).

LCMS: m/z (ESI): 362.0 $[M+H]^+$.

(*S*)-5-(3-(5-Chloro-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)-5-(1-methyl-1H-imidazol-2-yl)imidazolidine-2,4-dione (25) & its enantiomer (*R*)-5-(3-(5-chloro-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)-5-(1-methyl-1H-imidazol-2-yl)imidazolidine-2,4-dione (25')



Step 1: 1-(1-Methyl-1*H*-imidazol-2-yl)ethan-1-one (25-2)

To a solution of 1-methyl-1H-imidazole **25-1** (39 g, 475.01 mmol) in THF (350 mL) at -70 ℃ was added n-BuLi (1.6 N in hexane, 356 mL, 570.01 mmol) dropwise. After the addition, the resulting mixture was allowed to warm to 0 ℃ and stirred at this temperature for 30 min, and then the reaction was re-cooled to -70 ℃. Ethyl acetate (104.63 g, 1.19 mol) was added dropwise at -70 ℃. The reaction mixture was stirred at room temperature for 18 h then diluted with aqueous NH_4Cl (100 mL). The mixture was extracted with EtOAc (400 mL × 3). The organic layers were combined, washed with brine (400 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc/hexane = 1/10 to 1/2) to afford **25-2** (26 g, yield 44.09%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.14 (d, 1H), 7.04 (d, 1H), 4.00 (s, 3H), 2.66 (s, 3H).

Step 2: Tert-butyl 4-(1-methyl-1*H*-imidazol-2-yl)-4-oxobutanoate (25-3)

A solution of LDA (2 N in THF, 77.3 mL, 154.66 mmol) in THF (200 mL) was cooled to -78 ℃. A solution of **25-2** (16.0 g, 128.89 mmol) was added dropwise, then the resulting mixture was warmed to 0 ℃ and stirred for 30 min. The reaction mixture was re-cooled to -78 ℃ and tert-butyl 2-bromoacetate (25.14 g, 128.89 mmol) was added slowly. The reaction was stirred at room temperature overnight and quenched with saturated aqueous NH_4Cl (150 mL). The mixture was extracted with EtOAc (150 mL × 3). The combined organic layer was washed with brine (100 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc/hexane = 1/8 to 1/1) to afford **25-3** (13 g, yield 42.33%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.15-7.13 (m, 1H), 7.09-07.03 (s, 1H), 4.01-3.99 (m, 3H), 3.44-3.38 (m, 2H), 2.67-2.61 (m, 2H), 1.46-1.43 (m, 9H).

Step 3: Tert-butyl 3-(4-(1-methyl-1*H*-imidazol-2-yl)-2,5-dioxoimidazolidin-4-yl)propanoate (25-4)

A mixture of **25-3** (4.0 g, 16.79 mmol), $(NH_4)_2CO_3$ (13.70 g, 142.69 mmol), NaCN (2.23 g, 41.97 mmol. CAUTION! Sodium cyanide is highly toxic), EtOH (25 mL) and H_2O (25 mL) was added to sealed vessel and heated to 85 ℃ reacted for 18 h. The reaction mixture was diluted with water (100 mL). The mixture was extracted with n-BuOH (100 mL × 3). The combined organic layers were washed with brine (50 mL), concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH= 100/1 to 10/1) to afford **25-4** (700 mg, yield 13.52%).

LCMS: m/z (ESI): 309.4 $[M+H]^+$.

Step 4: 3-(4-(1-Methyl-1*H*-imidazol-2-yl)-2,5-dioxoimidazolidin-4-yl)propanoic acid (25a)

To a solution **25-4** (700 mg, 2.27 mmol) in 1,4-dioxane (20 mL) was added HCl/1,4-dioxane (4N, 20 mL, 80 mmol). The resulting mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated under reduced pressure to afford crude **25a** (700 mg, 2.78 mmol).

LCMS: m/z (ESI): 253.1 $[M+H]^+$.

Step 5: (*S*)-5-(3-(5-Chloro-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)-5-(1-methyl-1H-imidazol-2-yl)imidazolidine-2,4-dione (25) & (*R*)-5-(3-(5-chloro-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)-5-(1-methyl-1H-imidazol-2-yl)imidazolidine-2,4-dione (25')

A mixture of **25a** (110 mg, 436 μmol), **18h** (96.65 mg, 436 μmol), Et_3N (132 mg, 1.31 mmol) and HATU (166 mg, 436 μmol) in DMF (5.0 mL) was stirred at room temperature for 18 h. The reation mixture was purified by prep-HPLC to afford **25b** (30 mg, yield 15.1%) as a white solid. Compound **25b** (24 mg) was chirally separated by SFC to afford **25** (5.0 mg, yield 2.52%) and **25'** (5.0 mg, yield 2.52%).

Enantiomer **25** with shorter retention time:

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.16 (br, 1H), 8.59 (s, 1H), 7.91 (s, 1H), 7.77 (s, 1H), 7.19 (s, 1H), 6.84 (s, 1H), 4.85-4.81 (m, 2H), 4.71-4.67 (m, 2H), 3.54 (s, 3H), 2.59-2.54 (m, 2H), 2.45-2.38 (m, 2H).

LCMS: MS m/z (ESI): 456.1 $[M+H]^+$.

Chiral HPLC (CO_2/MeOH/DEA 60/40/0.04 2.8 mL/min, OD, 5 μm, 4.6*250 (Daicel)): Rt: 2.823 min, ee: 100%.

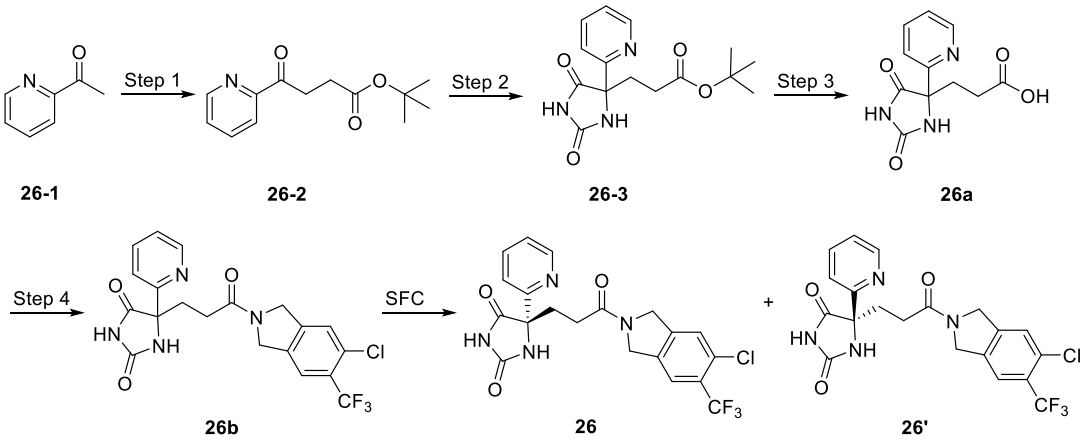
Enantiomer **25'** with longer retention time:

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.18 (br, 1H), 8.59 (s, 1H), 7.91 (s, 1H), 7.77 (s, 1H), 7.20 (s, 1H), 6.85 (s, 1H), 4.85-4.81 (m, 2H), 4.71-4.67 (m, 2H), 3.54 (s, 3H), 2.59-2.54 (m, 2H), 2.45-2.38 (m, 2H).

LCMS: MS m/z (ESI): 456.0 $[M+H]^+$.

Chiral HPLC (CO_2/MeOH/DEA 60/40/0.04 2.8 mL/min, OD, 5 μm, 4.6*250 (Daicel)): Rt: 3.878 min, ee: 96.48%.

(*S*)-5-(3-(5-Chloro-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)-5-(pyridin-2-yl)imidazolidine-2,4-dione (26) & its enantiomer (*R*)-5-(3-(5-chloro-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)-5-(pyridin-2-yl)imidazolidine-2,4-dione (26')



Step 1: Tert-butyl 4-oxo-4-(pyridin-2-yl)butanoate (26-2)

To a solution of 1-(pyridin-2-yl)ethanone **26-1** (24.2 g, 199 mmol) in THF (300 mL) at -70 ℃ was added LDA (2.0 M in THF, 120 mL, 240 mmol) dropwise. After the addition, the reaction mixture was stirred at this temperature for 30 min before tert-butyl 2-bromoacetate (39 g, 199 mmol) was added dropwise. Then the resulting mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with aqueous NH_4Cl (100 mL) and extracted with EtOAc (400 mL X 3). The organic layers were combined, washed with brine (400 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc/hexane = 1/10 to 1/2) to afford **26-2** (11.0 g, yield 23.4%) as a colorless oil.

LCMS: MS m/z (ESI): 236.1 $[M+H]^+$.

Step 2: Tert-butyl 3-(2,5-dioxo-4-(pyridin-2-yl)imidazolidin-4-yl)propanoate (26-3)

A mixture of **26-2** (4.7 g, 20.0 mmol), $(NH_4)_2CO_3$ (16.3 g, 170 mmol), NaCN (2.45 g, 50.00 mmol. CAUTION! Sodium cyanide is highly toxic), EtOH (25 mL) and H_2O (25 mL) was heated in an autoclave at 85 ℃ for 18 h. The resulting mixture was diluted with water (60 mL). The mixture was extracted with EtOAc (200 mL X 5). The organic layers were combined, washed with brine (200 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc/hexane = 1/10 to 1/1) to afford **26-3** (3.0 g, yield 49.2%) as a white solid.

LCMS: MS m/z (ESI): 306.1 $[M+H]^+$.

Step 3: 3-(2,5-Dioxo-4-(pyridin-2-yl)imidazolidin-4-yl)propanoic acid (26a)

To a solution of **26-3** (1.0 g, 3.28 mmol) in DCM (10 mL) was added HCl/1,4-dioxane (4.0 M, 30 mL). The resulting mixture was stirred at room temperature for 18 h. Then the reaction mixture was concentrated under reduced pressure. The residue was washed with Et_2O (10 mL), dried in vacuum to afford **26a** (800 mg, yield 98.01%) as a white solid.

LCMS: MS m/z (ESI): 248.2 $[M-1]^-$.

Step 4: (*S*)-5-(3-(5-Chloro-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)-5-(pyridin-2-yl)imidazolidine-2,4-dione (26) & (*R*)-5-(3-(5-chloro-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)-5-(pyridin-2-yl)imidazolidine-2,4-dione (26')

A mixture of **26a** (114 mg, 399 μmol), **18h** (78 mg, 302 μmol), HATU (152 mg, 399 μmol), TEA (162 mg, 1.60 mmol) and DMF (4.0 mL) was stirred at room temperature for 18 h. The reaction mixture was purified by prep-HPLC (Waters 2767/2545/2489, Waters Xbridge C18 10 μm OBD 19*250 mm, Mobile Phase A: 0.1% NH_4OH in water, Mobile Phase B: CH_3CN, Flow: 20 mL/min, Column temp: room temperature) to afford racemic mixture **26b** (60 mg), which was separated by SFC to afford **26** (15 mg, yield 8.30%) and **26'** (15 mg, yield 8.30%).

Enantiomer **26** with longer retention time:

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.87 (brs, 1H), 8.62 (dd, J = 4.8 and 0.8 Hz, 1H), 8.53 (s, 1H), 7.91-7.85 (m, 2H), 7.76 (d, J = 2.6 Hz, 1H), 7.54 (d, J = 4.0 Hz, 1H), 7.41-7.37 (m, 1H), 4.83-4.80 (m, 2H), 4.69-4.66 (m, 2H), 2.51-2.44 (m, 2H), 2.36-2.30 (m, 2H).

LCMS: MS m/z (ESI): 453.1 $[M+H]^+$.

Chiral HPLC (CO_2/EtOH/DEA 60/40/0.04 1.8 mL/min, IG, 3 μm, 3*100 (Daicel)): Rt: 2.713 min; Purity: 99.32%.

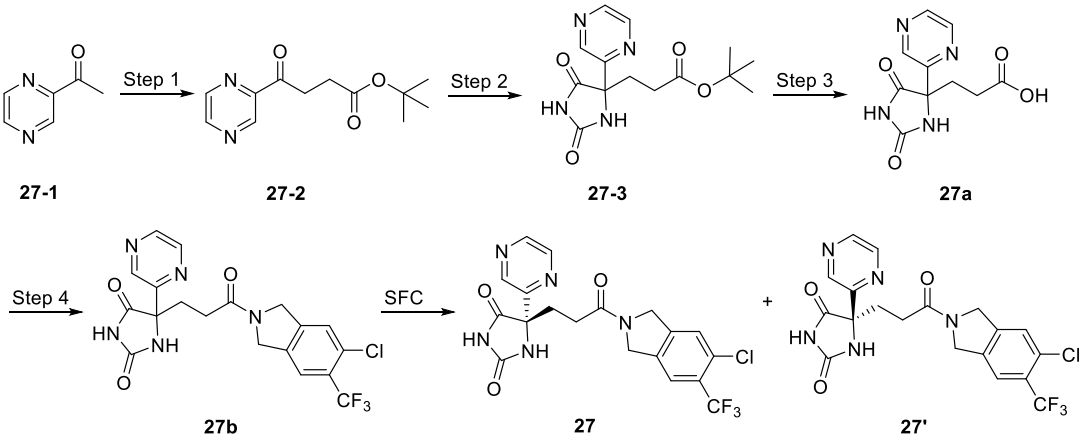
Enantiomer **26'** with shorter retention time:

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.87 (brs, 1H), 8.62 (dd, J = 4.8 and 0.8 Hz, 1H), 8.51 (s, 1H), 7.91-7.84 (m, 2H), 7.76 (d, J = 2.6 Hz, 1H), 7.54 (d, J = 4.0 Hz, 1H), 7.41-7.37 (m, 1H), 4.83-4.80 (m, 2H), 4.69-4.66 (m, 2H), 2.51-2.41 (m, 2H), 2.36-2.30 (m, 2H).

LCMS: MS m/z (ESI): 453.0 $[M+H]^+$.

Chiral HPLC (CO_2/EtOH/DEA 60/40/0.04 1.8 mL/min, IG, 3 μm, 3*100 (Daicel)): Rt: 1.852 min; Purity: 100%.

(*S*)-5-(3-(5-Chloro-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)-5-(pyrazin-2-yl)imidazolidine-2,4-dione (27) & its enantiomer (*R*)-5-(3-(5-chloro-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)-5-(pyrazin-2-yl)imidazolidine-2,4-dione (27')



Step 1: Tert-butyl 4-oxo-4-(thiazol-2-yl)butanoate (27-2)

To a solution of 1-(pyrazin-2-yl)ethan-1-one **27-1** (12.0 g, 98.3 mmol) in THF (200 mL) at -70 ℃ was added NaHMDs (2.0 M in THF, 49.2 mL, 98.3 mmol) dropwise. The resulting mixture was stirred at this temperature for 30 min before tert-butyl 2-bromoacetate (19.2 g, 98.3 mmol) was added dropwise. After addition, the reaction mixture was stirred at -20 ℃ for 1 h, then warmed to room temperature and stirred for 18 h. The resulting mixture was cooled to 0℃, then quenched with aqueous $NaHCO_3$ (200 mL). The mixture was extracted with EtOAc (300 mL X 4). The organic layers were combined, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc/hexane = 1/20 to 1/4) to afford **27-2** (13.5 g, yield 58.2%) as a colorless oil.

LCMS: MS m/z (ESI): 237.1 $[M+H]^+$

Step 2: Tert-butyl 3-(2,5-dioxo-4-(pyrazin-2-yl)imidazolidin-4-yl)propanoate (27-3)

A mixture of **27-2** (13.0 g, 55.0 mmol), $(NH_4)_2CO_3$ (44.9 g, 468 mmol) and NaCN (11.7 g, 220 mmol. CAUTION! Sodium cyanide is highly toxic) in EtOH (60 mL) and H_2O (60 mL) was heated to 120 ℃ in an autoclave and stirred for 18 h. The resulting mixture was diluted with water (100 mL). The mixture was extracted with EtOAc (200 mL X 3) and n-BuOH (200 mL X 3). The combined organic layers were washed with brine (200 mL) and concentrated under reduced pressure. The residue was purified by silica gel chromatography (MeOH/DCM = 1/100 to 1/30) to afford **27-3** (3.0 g, yield 17.80%) as a white solid.

LCMS: MS m/z (ESI): 307.1 $[M+H]^+$.

Step 3: 3-(2,5-Dioxo-4-(pyrazin-2-yl)imidazolidin-4-yl)propanoic acid (27a)

To a solution of **27-3** (900 mg, 2.94 mmol) in DCM (30 mL) was added HCl/1,4-dioxane (4.0 M, 30 mL, 120 mmol) dropwise. The reaction mixture was stirred at room temperature for 18 h. The resulting mixture was filtered in vacuum. The filter cake was collected, dried in vacuum to afford **27a** (600 mg, yield 81.62%) as a white solid.

LCMS: MS m/z (ESI): 249.0 $[M-H]^-$.

Step 4: (*S*)-5-(3-(5-Chloro-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)-5-(pyrazin-2-yl)imidazolidine-2,4-dione (27) & (*R*)-5-(3-(5-chloro-6-(trifluoromethyl)isoindolin-2-yl)-3-oxopropyl)-5-(pyrazin-2-yl)imidazolidine-2,4-dione (27')

A mixture of **27a** (130 mg, 519.56 μmol), **18h** (134 mg, 519.56 μmol), TEA (158 mg, 1.56 mmol) and HATU (198 mg, 519.56 μmol) in DMF (5 mL) was stirred at room temperature for 18 h. The resulting mixture was purified by prep-HPLC (Waters 2767/2545/2489, Waters Xbridge C18 10 um OBD 19*250 mm, Mobile Phase A: 0.1% NH_4OH in water, Mobile Phase B: CH_3CN, Flow: 20 mL/min, Column temp: room temperature) to afford racemic mixture **27b** (about 50 mg), which was further separated by SFC to afford two enantiomers **27** (16 mg, yield 6.79%) and **27'** (15 mg, yield 6.36%).

Enantiomer **27** with longer retention time:

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.01 (br, 1H), 8.82 (d, *J* = 1.2 Hz, 1H), 8.72-8.70 (m, 1H), 8.67 (d, *J* = 2.4 Hz, 1H), 8.57 (brs, 1H), 7.90 (d, *J* = 6.4 Hz, 1H), 7.75 (d, *J* = 4.8 Hz, 1H), 4.84-4.80 (m, 2H), 4.69-4.65 (m, 2H), 2.49-2.46 (m, 2H), 2.37-2.33 (m, 2H).

Chiral HPLC (CO_2/MeOH/DEA 60/40/0.04 2.8 mL/min, AY, 5 μm, 4.6*250 (Daicel)): Rt: 4.485 min; ee: 99.43%.

LCMS: MS m/z (ESI): 454.1 $[M+H]^+$.

Enantiomer **27'** with shorter retention time:

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.05 (brs, 1H), 8.83 (d, J = 1.2 Hz, 1H), 8.73-8.71 (m, 1H), 8.67 (d, J = 2.4 Hz, 1H), 8.65 (br, 1H), 7.90 (d, J = 6.4 Hz, 1H), 7.75 (d, J = 4.8 Hz, 1H), 4.84-4.80 (m, 2H), 4.69-4.65 (m, 2H), 2.49-2.46 (m, 2H), 2.37-2.33 (m, 2H).

Chiral HPLC (CO_2/MeOH/DEA 60/40/0.04 2.8 mL/min, AY, 5 μm, 4.6*250 (Daicel)): Rt: 2.652 min, ee: 100%.

LCMS: MS m/z (ESI): 454.1 $[M+H]^+$.

LC-MS/MS instrumentation and general conditions

LC-MS/MS for in vitro and in vivo sample bioanalysis consisted of an HPLC system (Shimadzu, Japan) equipped with a binary solvent manager, an auto sampler and an AB4000 triple quadrupole mass spectrometer (AB SCIEX) with electrospray ionization (ESI) source. Data acquisition and analysis were performed using Analyst software (AB SCIEX). A Xtimate C18 column (30 x 2.1mm i.d., 1.8 μm; Welch, China) thermostats at 35 °C. The flow rate was set to 0.3 mL/min. The mobile phase consisted of water with 0.2% formic acid (A) and acetonitrile (B). Gradient elution started from 15% (B), followed by a linear gradient to 85% (B) over 0.6 min and held for another 0.6 min then zoomed to 15% (B) in the next 0.1 min, and finally re-equilibrated to 15% (B) in 0.9 min. MS detection was performed in a positive ESI mode with the source temperature at 550 ℃ and ion spray voltage at 5.5 kV.

Reactive metabolite identification of GSH adduct in human liver microsomes

Microsomal incubation was carried out in PBS solution (pH = 7.4, 100 mM) in a total volume of 400 μL containing human liver microsomes (1 mg/mL), the test compound (30 μM), NADPH (1 mM) and $MgCl_2$ (3 mM). The mixture was pre-incubated at 37 ℃ for 5 min, and then GSH (5 mM) was added to start the reaction. After a 60-min incubation at 37 ℃, 0.8 mL of acetonitrile was added to terminate the reaction. The sample was then centrifuged at 20,879 × *g* for 10 min. The resulting supernatant was dried in vacuum and the residue was redissolved with 200 μL of water-acetonitrile solution (v/v, 3:1). After centrifuging again (20,879 × *g* for 10 min), 10 μL of the supernatant was submitted to LC-MS for metabolite identification and profiling. GSH adduct formation between Diclofenac (10 μM) and GSH was used as positive control.

Liver microsome stability

Stability of test compound in mouse, rat, and human liver microsome was determined according to method described in literatures.[3, 4]

CYP inhibition assay

The inhibitory IC_{50} values of tested compounds for five major P450 enzymes were determined in human microsomal experiments according to method described in literatures.[5, 6]

Plasma protein binding

Mouse, rat, and human plasma protein bonding of test compound was determined via S9 Rapid Equilibrium Dialysis (RED) system according to method described in literatures.[7, 8]

hERG assay

hERG inhibitory activity of test compound was determined by the Automated Patch-Clamp system according to method described in literature.[9]

Oral PK assay

The experimental protocol used in this study was approved by IACUC. The experiments were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals and complied with the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments). All animals were treated in accordance with Institutional Guide for the Care and Use of Laboratory Animals. Sprague Dawley (SD) rats and C57 micewere purchased from Shanghai SLAC Laboratory Animal Co., LTD (SYXK 2003-0029). Beagle dogs and cynomolgus monkeys were obtained from Suzhouxishan Zhongke Laboratory Animal Co., Ltd. (Suzhou, China).

Rat PK: formulation (5% DMSO + 5% tween 80 + 90% saline). Two groups of animals (2 male & 2 female in each group) were fasting overnight. Briefly, one group of rats were administrated by oral gavage at a dose of 2.0 mg/kg compound **18** and the other group of rats were administrated by intravenous injection at a dose of 1.0 mg/kg compound **18**. Serial blood samples (0.2 mL) were collected via the posterior orbital venous plexus into di-potassium ethylenediaminetetraacetic acid (K2 EDTA) blood collection tube. Blood samples were collected at pre-dose and 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 11.0, 24.0 h after oral gavage. And blood samples were collected at pre-dose and 5 min, 15 min, 0.5, 1.0, 2.0, 4.0, 8.0, 11.0, 24.0 h after intravenous injection administration. The blood samples were centrifuged at 4 °C (3500 rpm) for 10 min to separate the plasma and stored at -20 °C until analyzed by validated liquid chromatography-tandem mass spectrometry (LC/MS/MS) method. The whole procedure from blood collection to centrifugation was performed under ice condition.

Mouse PK: formulation (5% DMSO + 5% tween 80 + 90% saline). Two groups of animals (9 female in each group) were fasting overnight. Briefly, one group of mice were administrated by

oral gavage at a dose of 2.0 mg/kg compound **18** and the other group of mice were administrated by intravenous injection at a dose of 1.0 mg/kg compound **18**. Serial blood samples (0.2 mL) were collected via the posterior orbital venous plexus into di-potassium ethylenediaminetetraacetic acid (K2 EDTA) blood collection tube. Blood samples were collected at 15 min, 30 min, 1.0, 2.0, 4.0, 6.0, 8.0, 11.0, 24.0 h after oral gavage. And blood samples were collected at 5 min, 15 min, 30 min, 1.0, 2.0, 4.0, 8.0, 11.0, 24.0 h after intravenous injection administration. The blood samples were centrifuged at 4 °C (10000 rpm) for 10 min to separate the plasma and stored at -20 °C until analyzed by validated liquid chromatography-tandem mass spectrometry (LC/MS/MS) method. The whole procedure from blood collection to centrifugation was performed under ice condition.

Dog PK: formulation (5%DMSO +20%PG +20%PEG400 +55% saline). Two groups of animals (3 male in each group) were fasting overnight. Monkey blood samples (1.0 mL) were collected via the vein of the forelimbs at the time points of pre-dose and post-dose at 0.083 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h by intravenous injection administration (0.5 mpk of compound **18**) and pre-dose and post-dose at 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h by oral gavage (2 mpk of compound **18**), respectively. The blood samples were centrifuged at 4 °C (2200 G) for 10 min to separate the plasma and stored at -80 °C until analyzed by validated liquid chromatography-tandem mass spectrometry (LC/MS/MS) method. The whole procedure from blood collection to centrifugation was performed under ice condition.

Monkey PK: formulation (5%DMSO + 20%PG + 20%PEG400 + 55% saline). Two groups of animals (3 male in each group) were fasting overnight. Monkey blood samples (1.0 mL) were collected via the vein of the forelimbs at the time points of pre-dose and post-dose at 0.083 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h by intravenous injection administration (0.5 mpk of compound **18**) and pre-dose and post-dose at 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h by oral gavage (2 mpk of compound **18**), respectively. The blood samples were centrifuged at 4 °C (2200 G) for 10 min to separate the plasma and stored at -80 °C until analyzed by validated liquid chromatography-tandem mass spectrometry (LC/MS/MS) method. The whole procedure from blood collection to centrifugation was performed under ice condition.

Data analysis: The pharmacokinetic parameters were obtained by non-compartmental analysis of plasma concentration (determined by LC/MS/MS) *vs*. time point. The peak concentration (C_{max}) and time for C_{max} was recorded directly from experimental observations.

The area under curve (AUC_{0-t}) from time zero to the last sampling time was calculated using a combination of linear and log trapezoidal summations.

REFERENCES

1. Shiozaki, M.; Maeda, K.; Miura, T.; Kotoku, M.; Yamasaki, T.; Matsuda, I.; Aoki, K.; Yasue, K.; Imai, H.; Ubukata, M.; Suma, A.; Yokota, M.; Hotta, T.; Tanaka, M.; Hase, Y.; Haas, J.; Fryer, A. M.; Laird, E. R.; Littmann, N. M.; Andrews, S. W.; Josey, J. A.; Mimura, T.; Shinozaki, Y.; Yoshiuchi, H.; Inaba, T. Discovery of (1S,2R,3R)-2,3-dimethyl-2-phenyl-1-sulfamidocyclopropanecarboxylates: novel and highly selective aggrecanase inhibitors. *J. Med. Chem.* **2011**, *54*, 2839– 2863.

2. Brebion, F.; Gosmini, R.; Deprez, P.; Varin, M.; Peixoto, C.; Alvey, L.; Jary, H.; Bienvenu, N.; Triballeau, N.; Blanque, R.; Cottereaux, C.; Christophe, T.; Vandervoort, N.; Mollat, P.; Touitou, R.; Leonard, P.; De Ceuninck, F.; Botez, I.; Monjardet, A.; van der Aar, E.; Amantini, D. Discovery of GLPG1972/S201086, a potent, selective, and orally bioavailable ADAMTS-5 inhibitor for the treatment of osteoarthritis. *J. Med. Chem*. **2021**, *64*, 2937-2952.

3. Masimirembwa, C. M.; Bredberg, U..; Andersson, T. B. Metabolic stability for drug discovery and development. *Clin*. *Pharmacokinet*. **2003**, *42*, 515-528.

4. Davies, B.; Morris, T. Physiological parameters in laboratory animals and humans. *Pharm*. *Res*. **1993**, *10*, 1093-1095.

5. Li, G.; Huang, K.; Nikolic, D.; van Breemen, R. B. High-throughput cytochrome P450 cocktail inhibition assay for assessing drug-drug and drug-botanical interactions. *Drug Metab*. *Dispos*. **2015**, *43*, 1670–1678.

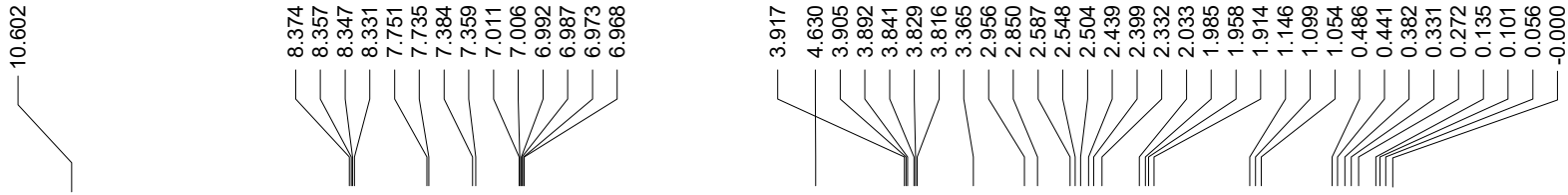
6. FDA Guidance for Industry: Drug Interaction Studies —Study design, data analysis, and implications for dosing and labeling, **2006**.

7. Wan, H.; Rehngren, M. High throughput screening of protein binding by equilibrium dialysis combined with liquid chromatography and mass spectrometry. *J*. *Chromatogr*. *A* **2006**, *1102*, 125-134.

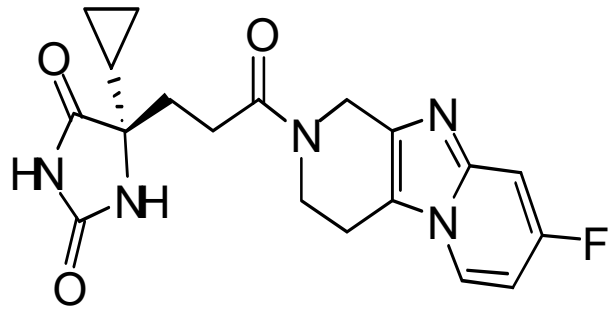
8. Waters, N. J.; Jones, R.; Williams, G.; Sohal, B. Validation of a rapid equilibrium dialysis approach for the measurement of plasma protein binding. *J*. *Pharm*. *Sci*. **2008**, *97*, 4586-4595.

9. Kutchinsky, J.; Friis, S.; Asmild, M.; Taboryski, R.; Pedersen, S.; Vestergaard, R. K.; Jacobsen, R. B.; Krzywkowski, K.; Schrøder,R. L.; Ljungstrøm, T.; Hélix, N.; Sørensen, C. B.;

Bech, M.; Willumsen, N. J. Characterization of potassium channel modulators with QPatch^{TM} automated patch-clamp technology: system characteristics and performance. *Assay Drug Dev. Technol*. **2003**, *1*, 685-693.

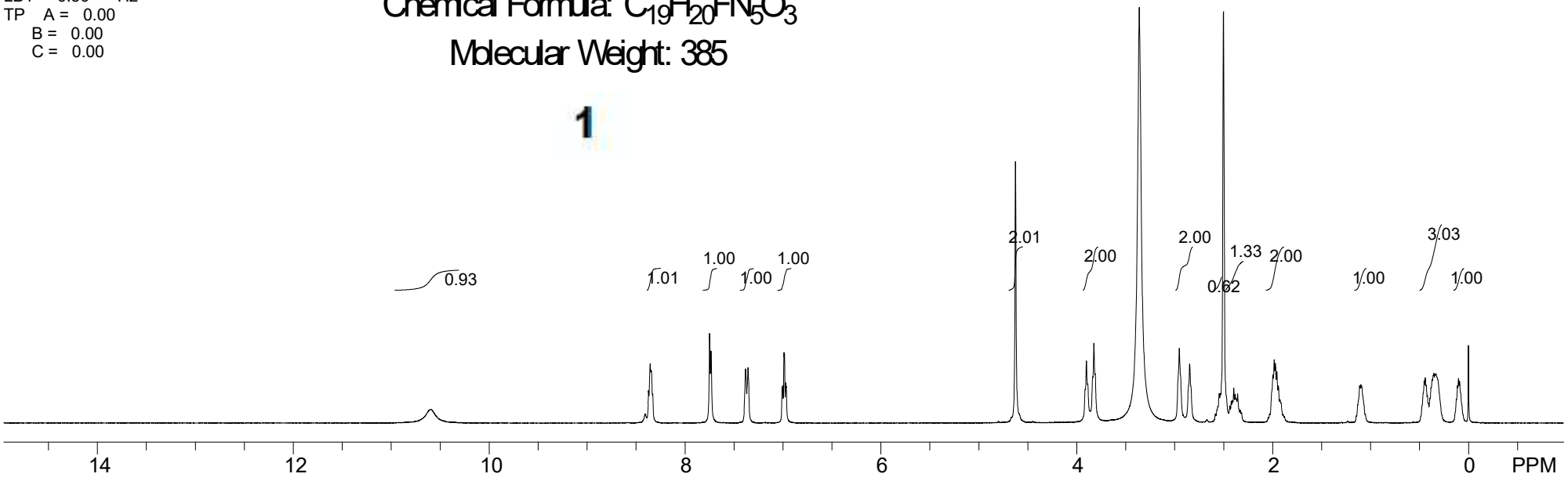


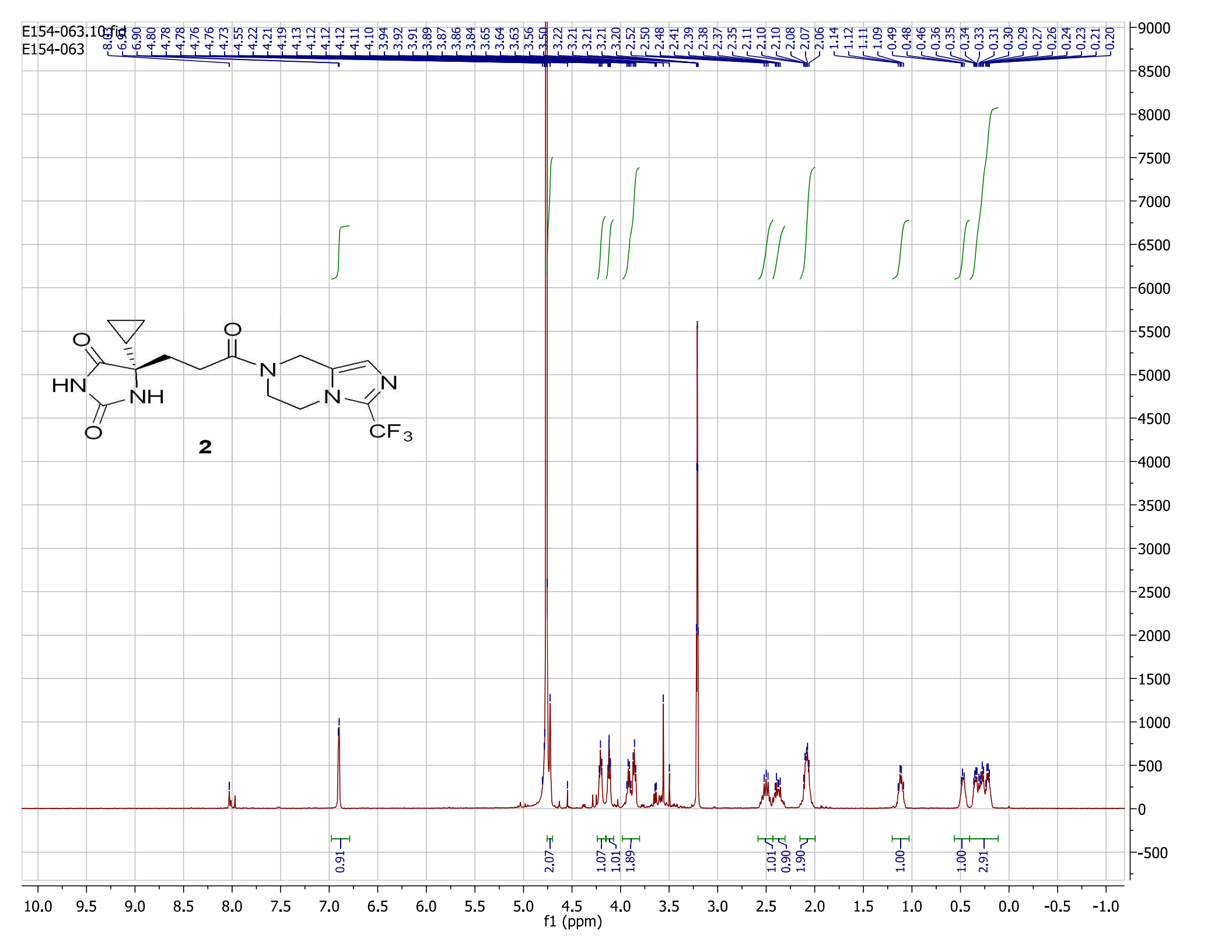
P:\PUBLIC\NMR_NUTS\New-nuts\nuts\DATA\$NMR-8075100-A1_1.1
, ,
16:22:44.244 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 32
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2471.0762 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00

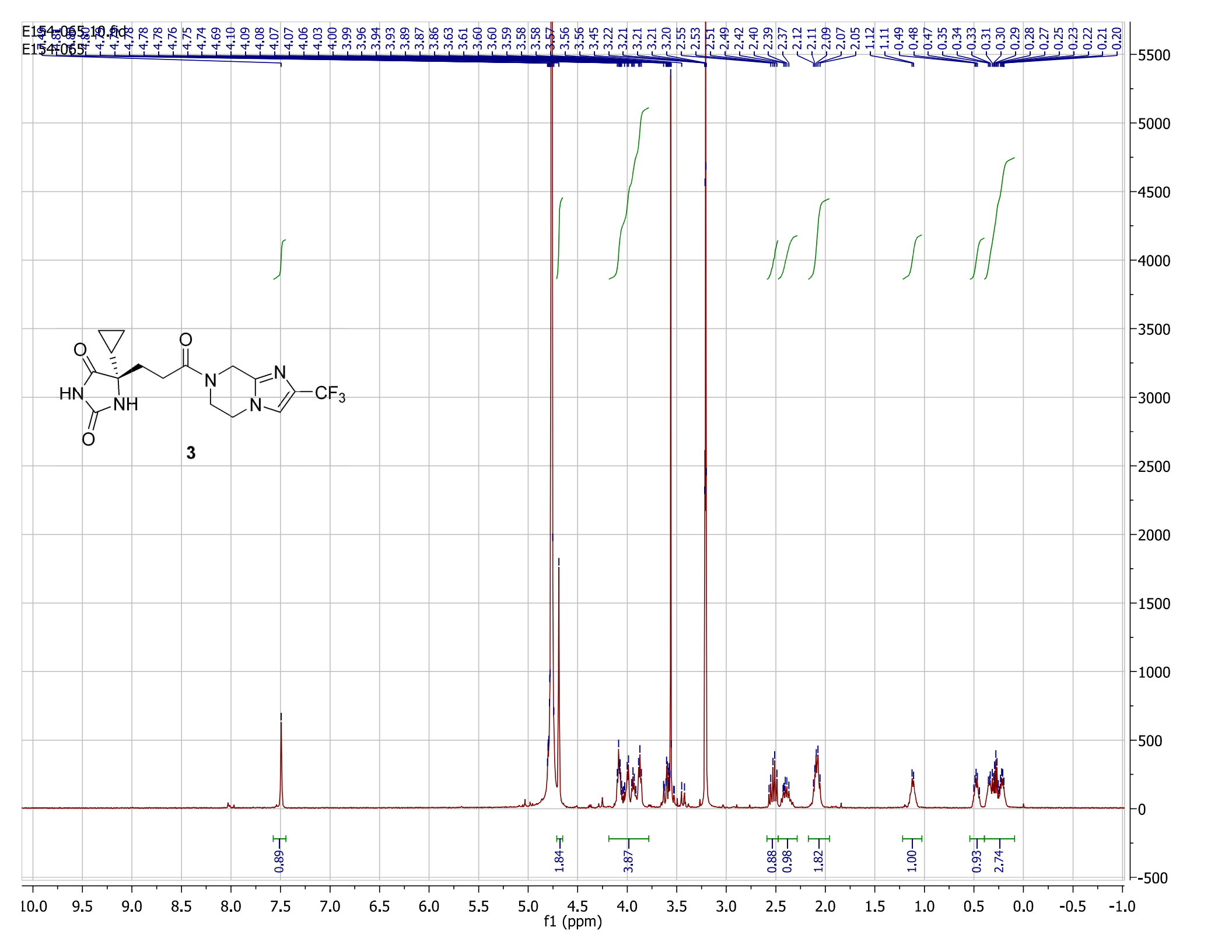


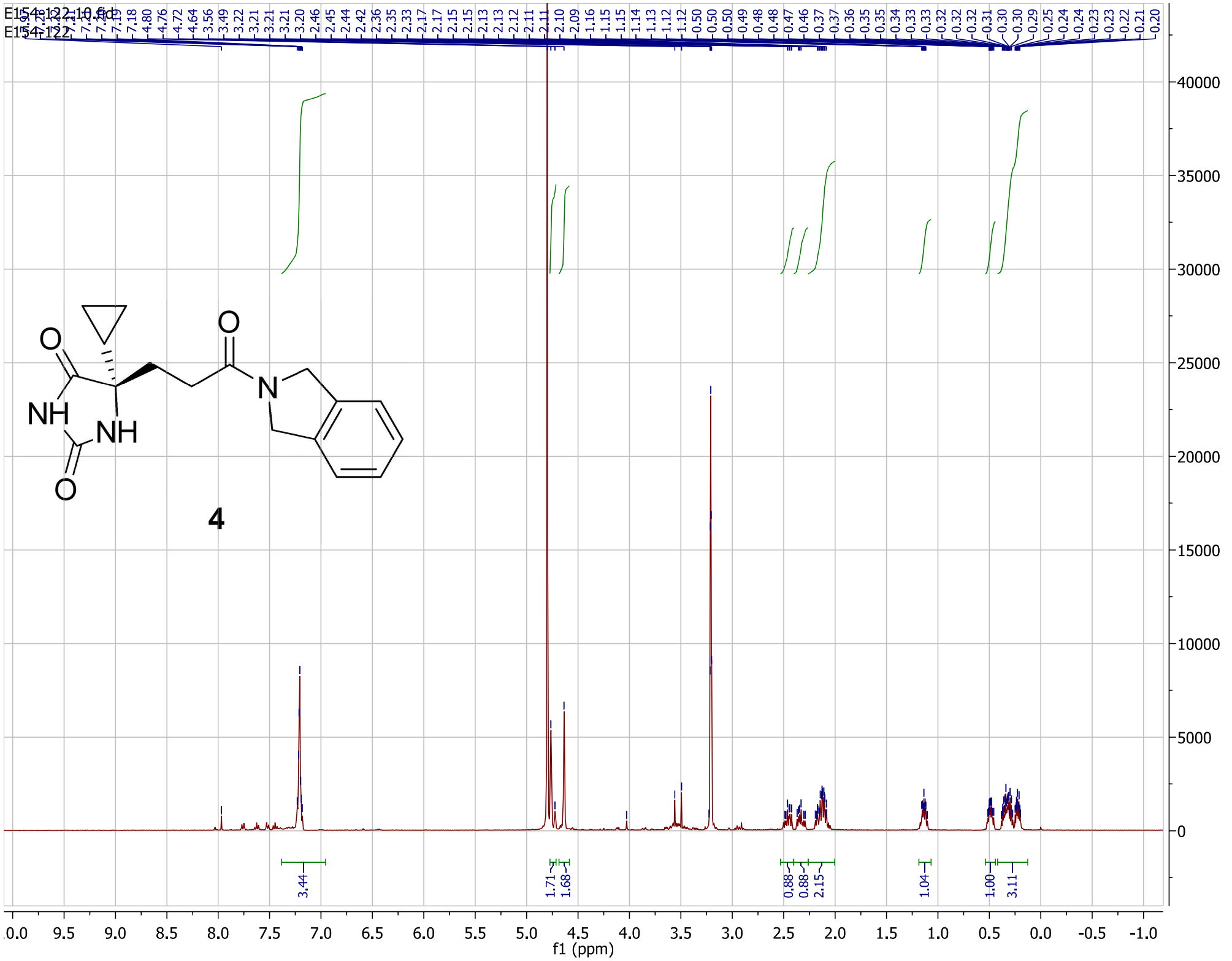
Chemical Formula: $C_{19}H_{20}FN_5O_3$
Molecular Weight: 385

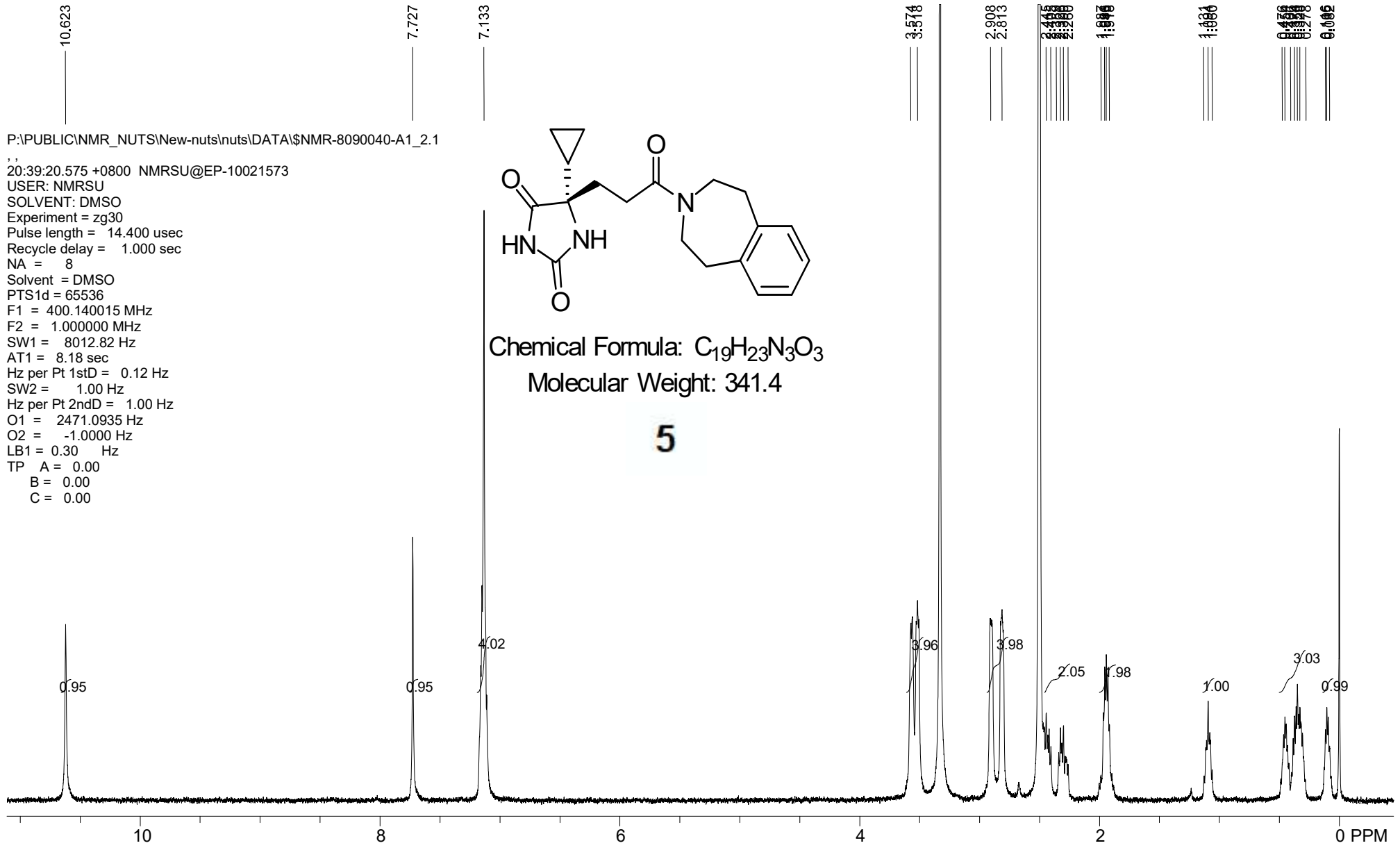
1

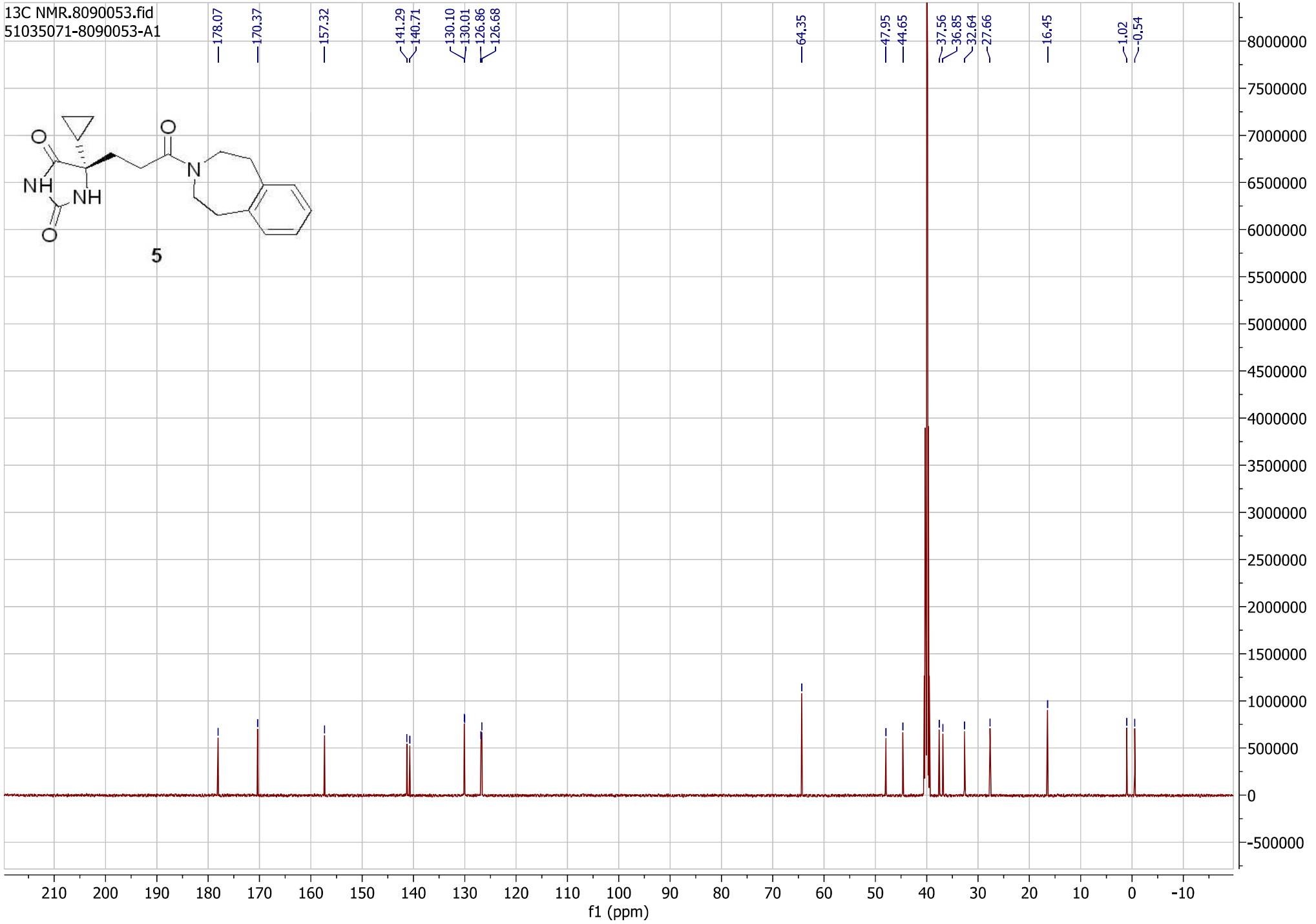


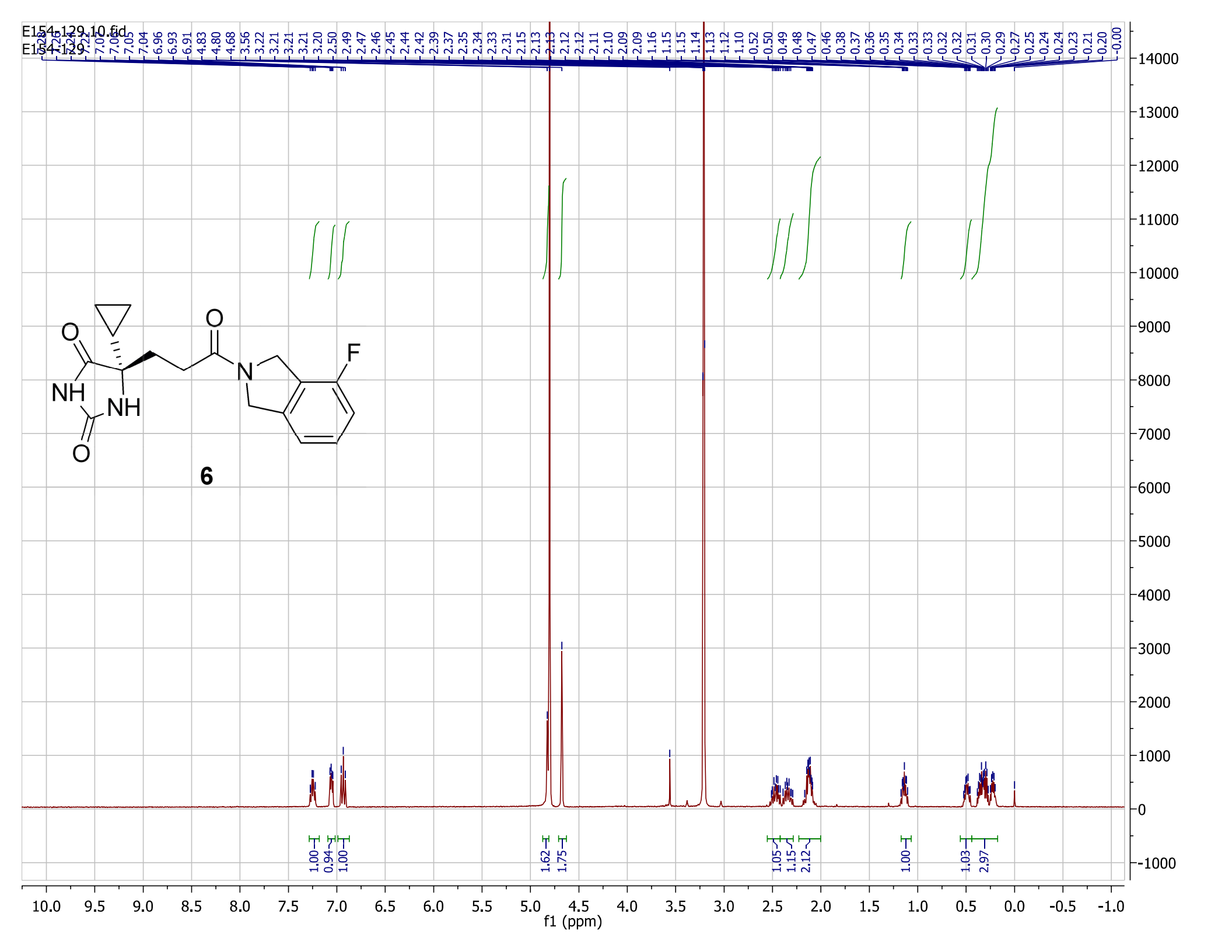

E154-063.10.fid
E154-063
HN
NH
O
O
O
N
N
N
CF3
2
f1 (ppm)

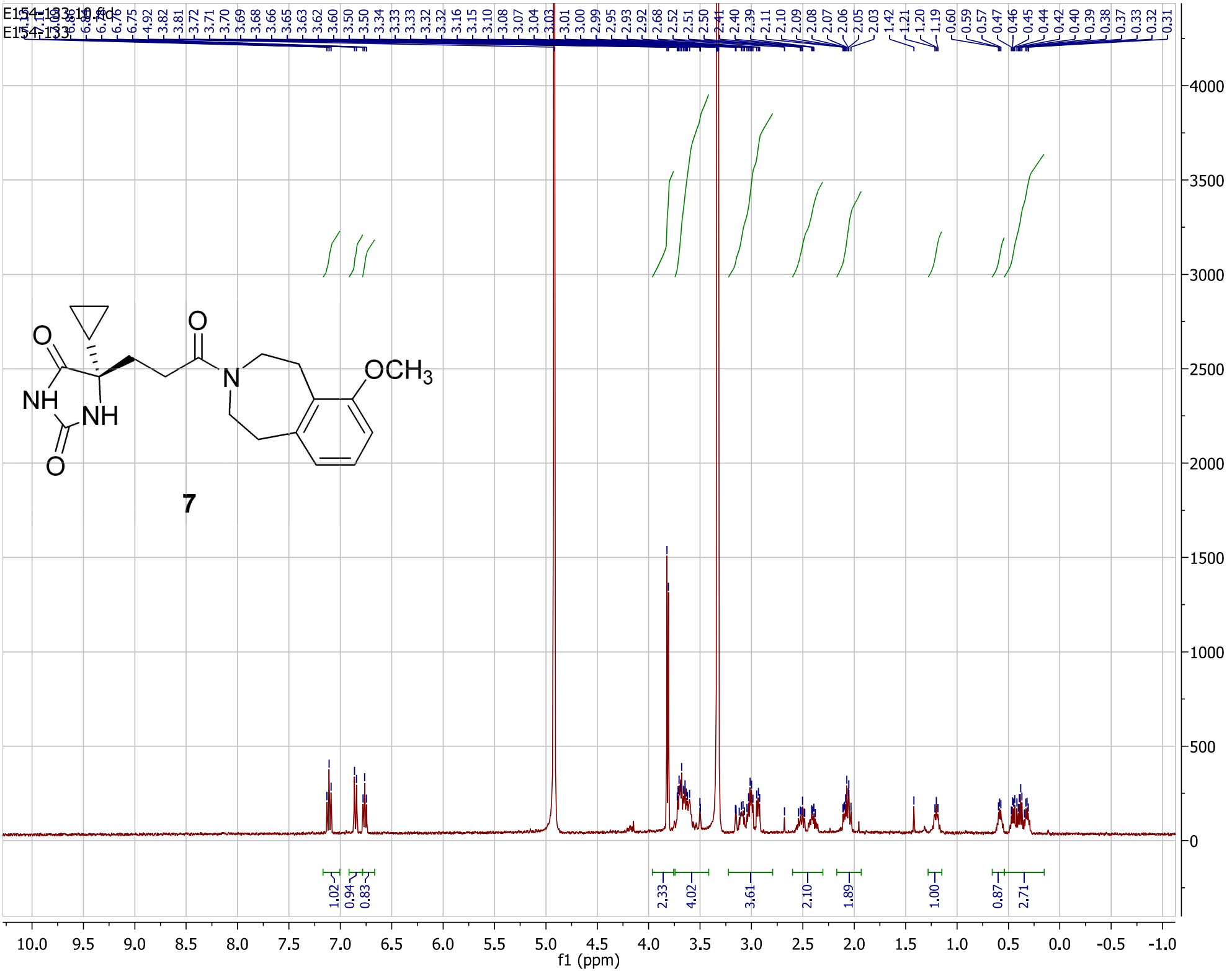

E154-065-10.fid
E154-065
HN
NH
O
O
O
N
N
N
CF3
3
0.89
1.84
3.87
0.88
0.98
1.82
1.00
0.93
2.74
f1 (ppm)

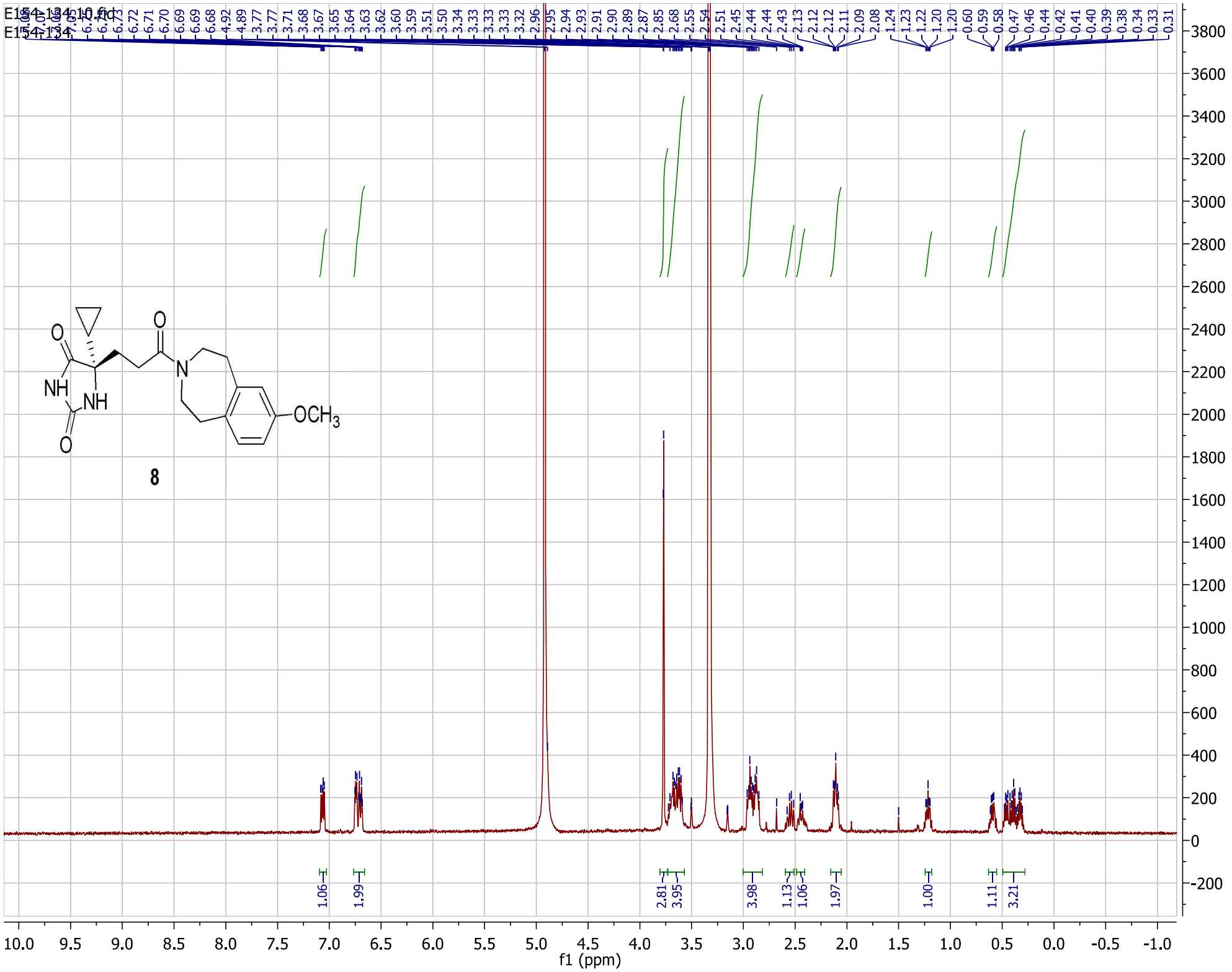

E154-122-10.fid
E154-122
O
O
NH
NH
O
N
4
3.44
1.71
1.68
0.88
0.88
2.15
1.04
1.00
3.11
f1 (ppm)

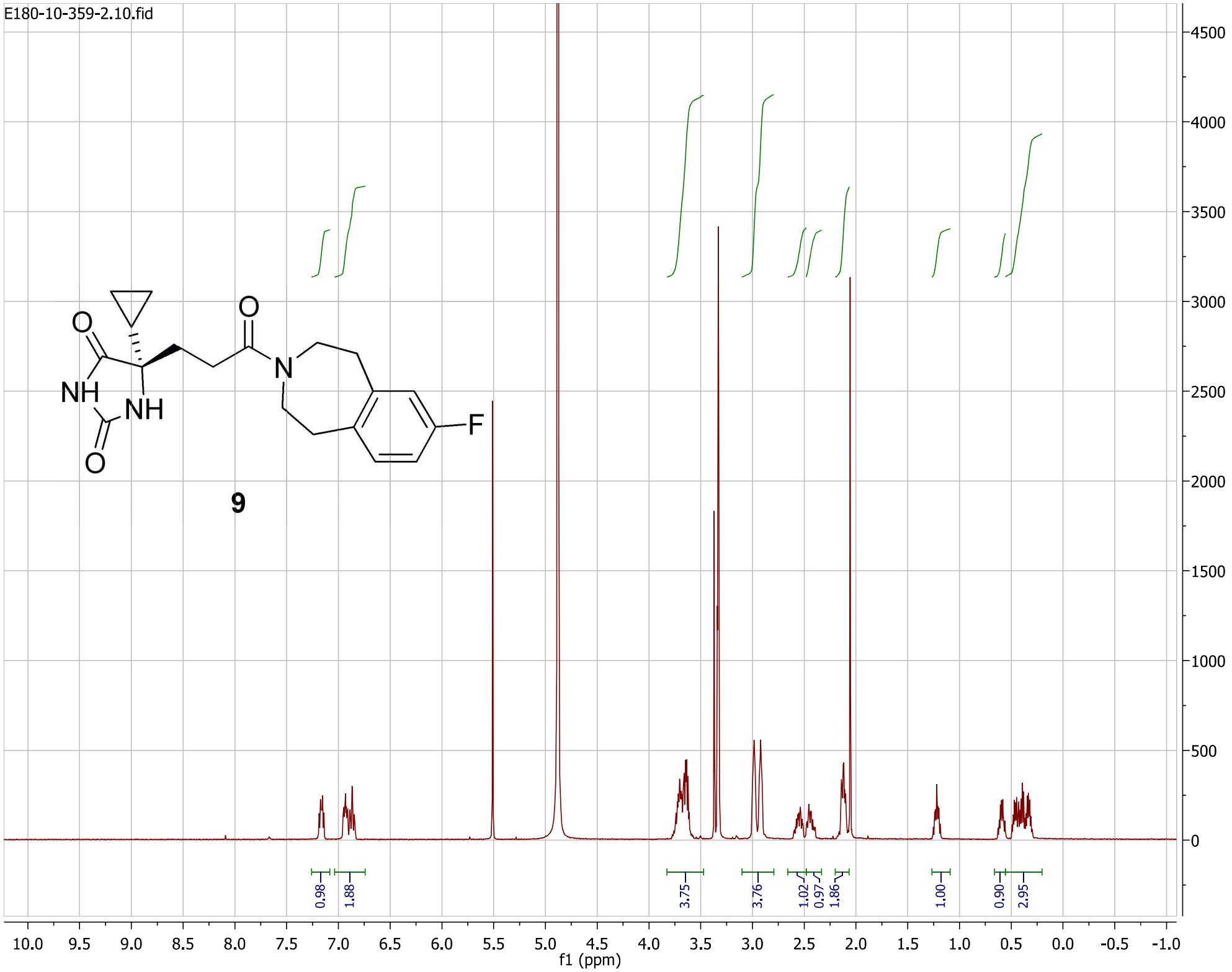

10.623
7.727
7.133
3.574
2.908
2.813
P:\PUBLIC\NMR_NUTS\New-nuts\nuts\DATA\$NMR-8090040-A1_2.1
, ,
20:39:20.575 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2471.0935 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
HN
NH
O
N
Chemical Formula: C19H23N3O3
Molecular Weight: 341.4
5
0.95
0.95
4.02
3.96
3.98
2.05
1.98
1.00
3.03
0.99
10
8
6
4
2
0 PPM

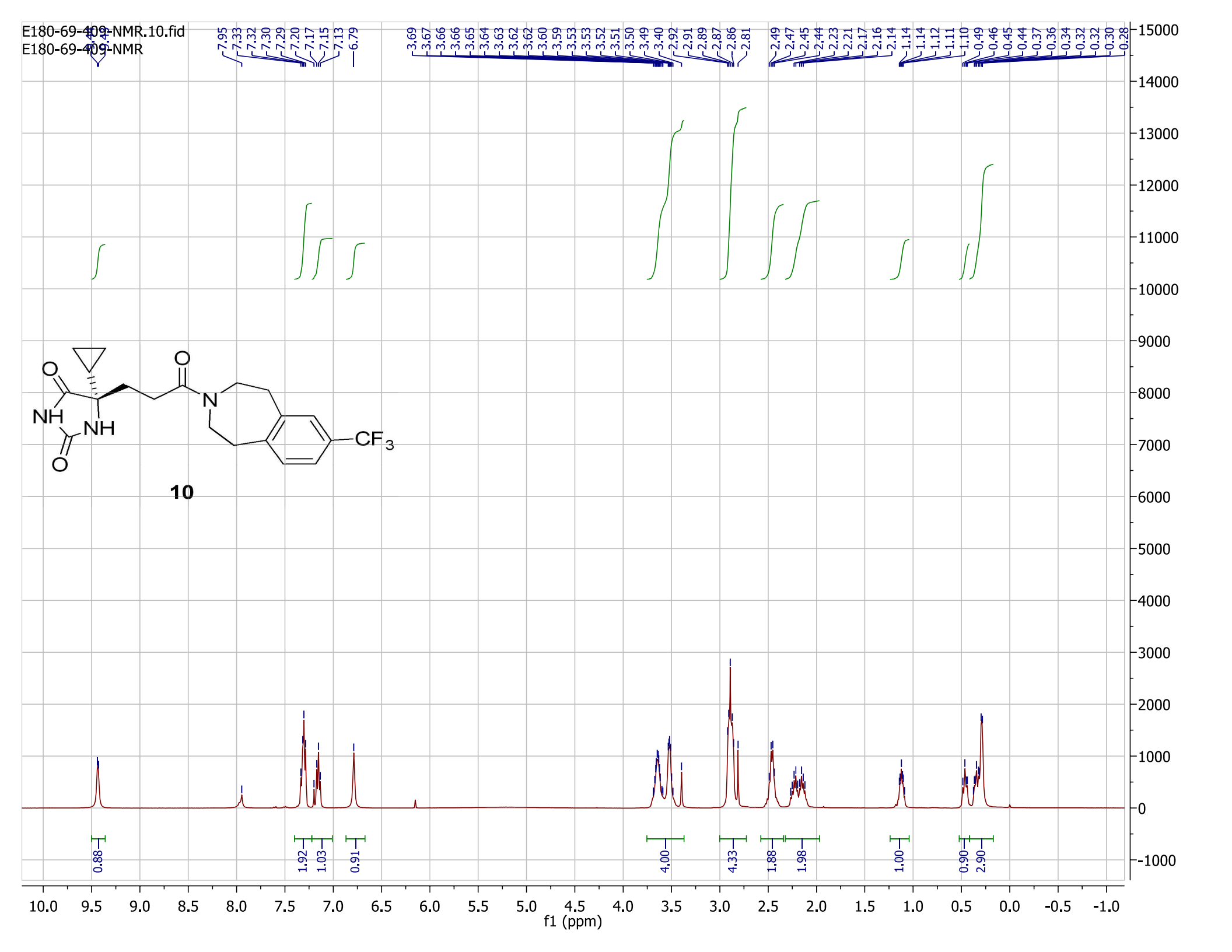

13C NMR.8090053.fid
51035071-8090053-A1
178.07
170.37
157.32
141.29
140.71
130.10
130.01
126.86
126.68
64.35
47.95
44.65
37.56
36.85
32.64
27.66
16.45
1.02
0.54
O
NH
NH
O
O
N
5
8000000
7500000
7000000
6500000
6000000
5500000
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
-500000
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

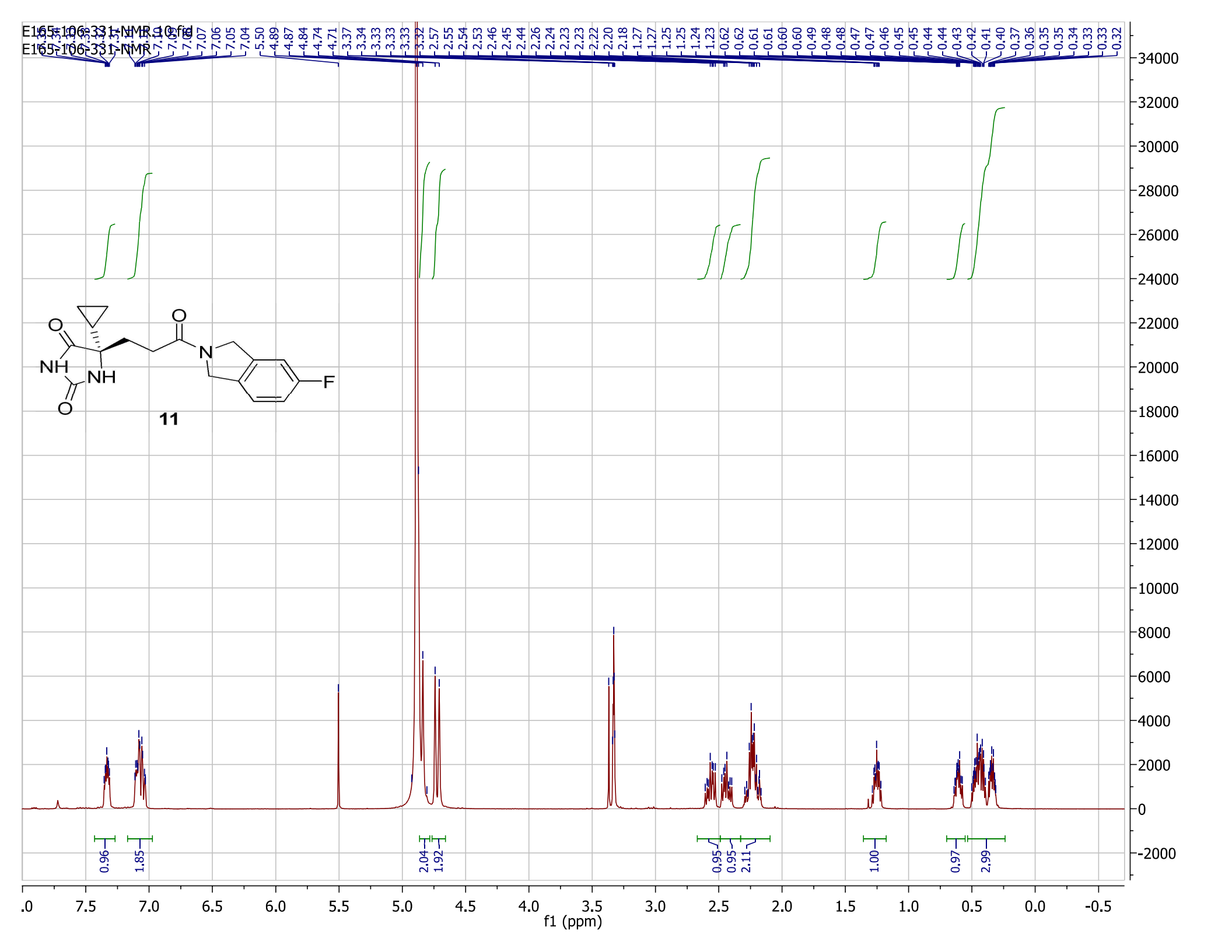

E154-129-10.fid
E154-129
O
O
NH
NH
O
N
F
6
1.00
0.94
1.00
1.62
1.75
1.05
1.15
2.12
1.00
1.03
2.97
f1 (ppm)

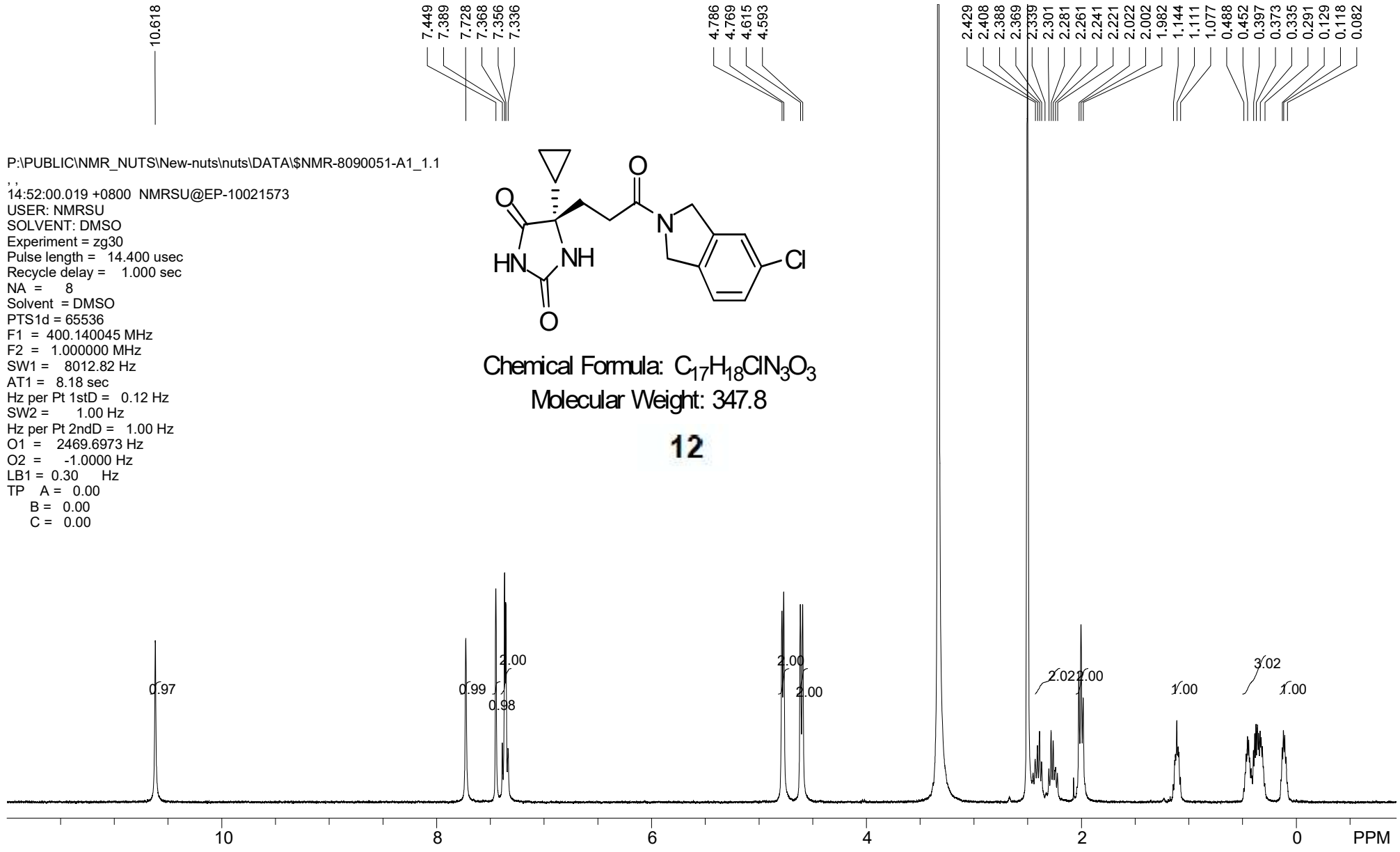

E154-133-1H.fid
E154-133
6.78
6.76
6.75
4.92
3.82
3.81
3.72
3.71
3.70
3.69
3.68
3.66
3.65
3.63
3.62
3.60
3.50
3.50
3.34
3.33
3.33
3.32
3.32
3.16
3.15
3.10
3.08
3.07
3.04
3.03
3.01
3.00
2.99
2.95
2.93
2.92
2.68
2.52
2.51
2.50
2.41
2.40
2.39
2.11
2.10
2.09
2.08
2.07
2.06
2.05
2.03
1.42
1.21
1.20
1.19
0.60
0.59
0.57
0.47
0.46
0.45
0.44
0.42
0.40
0.39
0.38
0.37
0.33
0.32
0.31
O
O
NH
NH
O
N
OCH3
7
4000
3500
3000
2500
2000
1500
1000
500
0
1.02
0.94
0.83
2.33
4.02
3.61
2.10
1.89
1.00
0.87
2.71
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)

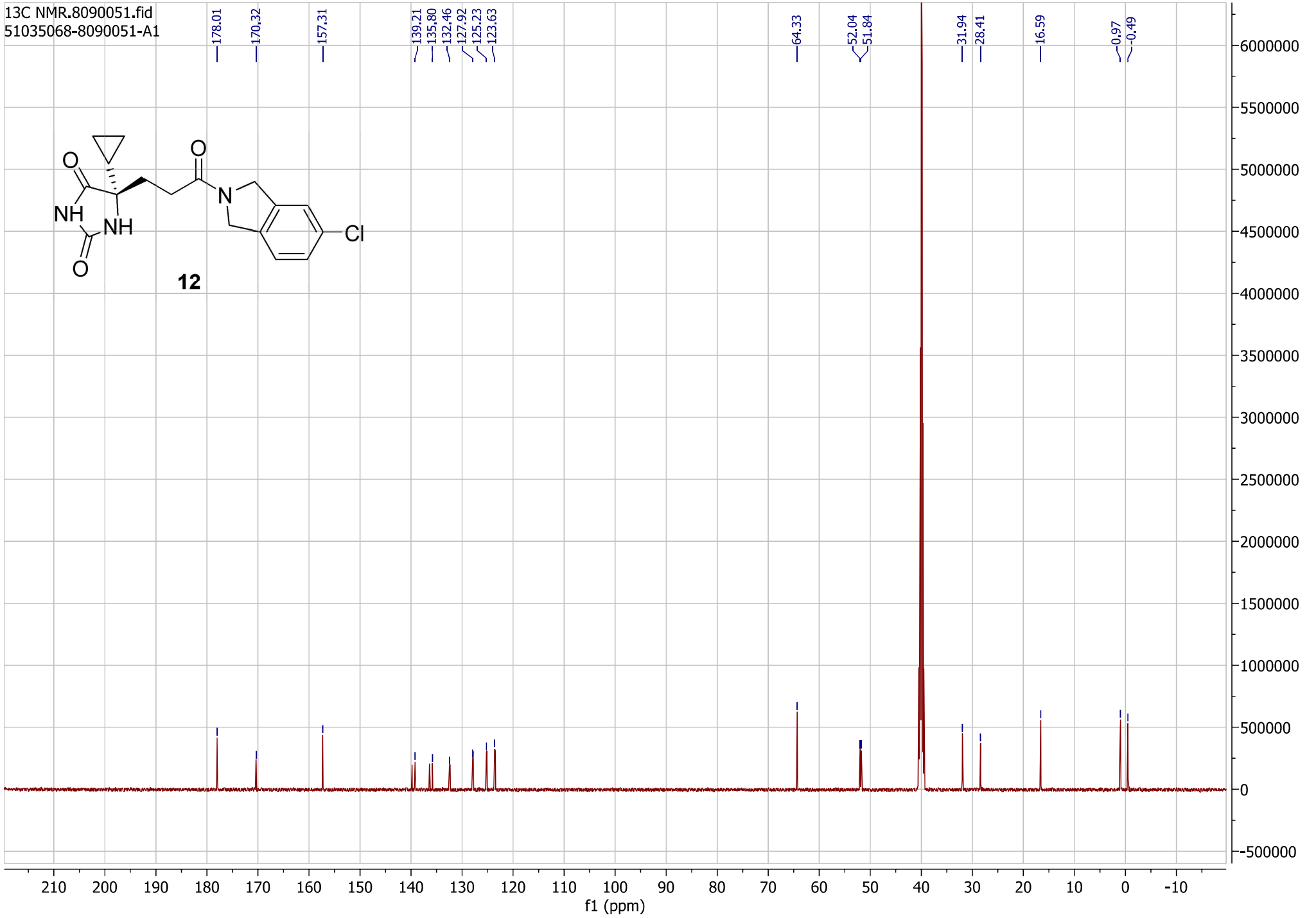

E154-134-10.fid
E154-134-1.fid
O
O
NH
NH
O
N
OCH3
8
1.06
1.99
2.81
3.95
3.98
1.13
1.06
1.97
1.00
1.11
3.21
f1 (ppm)


E180-10-359-2.10.fid
O
O
NH
NH
O
N
F
9
0.98
1.88
3.75
3.76
1.02
0.97
1.86
1.00
0.90
2.95
f1 (ppm)


E180-69-409-NMR.10.fid
E180-69-409-NMR
7.95 7.33 7.32 7.30 7.29 7.20 7.17 7.15 7.13 6.79
3.69 3.67 3.66 3.66 3.65 3.64 3.63 3.62 3.62 3.60 3.59 3.53 3.53 3.52 3.51 3.50 3.49 3.40 2.92 2.91 2.89 2.87 2.86 2.81
2.49 2.47 2.45 2.44 2.23 2.21 2.17 2.16 2.14 1.14 1.14 1.12 1.11 1.10 0.49 0.46 0.45 0.44 0.37 0.36 0.34 0.32 0.32 0.30 0.28
NH
NH
O
O
O
N
CF3
10
0.88
1.92
1.03
0.91
4.00
4.33
1.88
1.98
1.00
0.90
2.90
f1 (ppm)

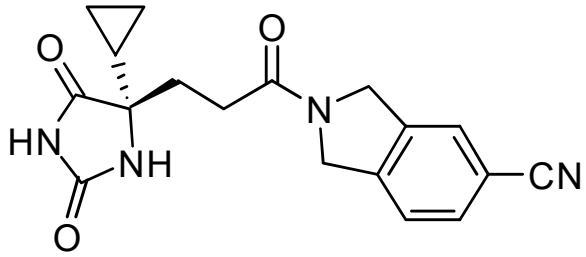

E165-106-331-NMR-10.fid
E165-106-331-NMR
O
NH
NH
O
O
N
F
11
0.96
1.85
2.04
1.92
0.95
0.95
2.11
1.00
0.97
2.99
f1 (ppm)


10.618
7.449
7.389
7.728
7.368
7.356
7.336
4.786
4.769
4.615
4.593
2.429
2.408
2.388
2.369
2.339
2.301
2.281
2.261
2.241
2.221
2.022
2.002
1.982
1.144
1.111
1.077
0.488
0.452
0.397
0.373
0.335
0.291
0.129
0.118
0.082
P:\PUBLIC\NMR_NUTS\New-nuts\nuts\DATA\$NMR-8090051-A1_1.1
, ,
14:52:00.019 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.140045 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2469.6973 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
O
O
HN
NH
O
N
Cl
Chemical Formula: C17H18ClN3O3
Molecular Weight: 347.8
12
0.97
0.99
2.00
0.98
2.00
2.00
2.02
2.00
1.00
3.02
1.00
10
8
6
4
2
0
PPM


13C NMR.8090051.fid
51035068-8090051-A1
178.01
170.32
157.31
139.21
135.80
132.46
127.92
125.23
123.63
64.33
52.04
51.84
31.94
28.41
16.59
0.97
-0.49
O
NH
NH
O
O
N
Cl
12
6000000
5500000
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
-500000
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

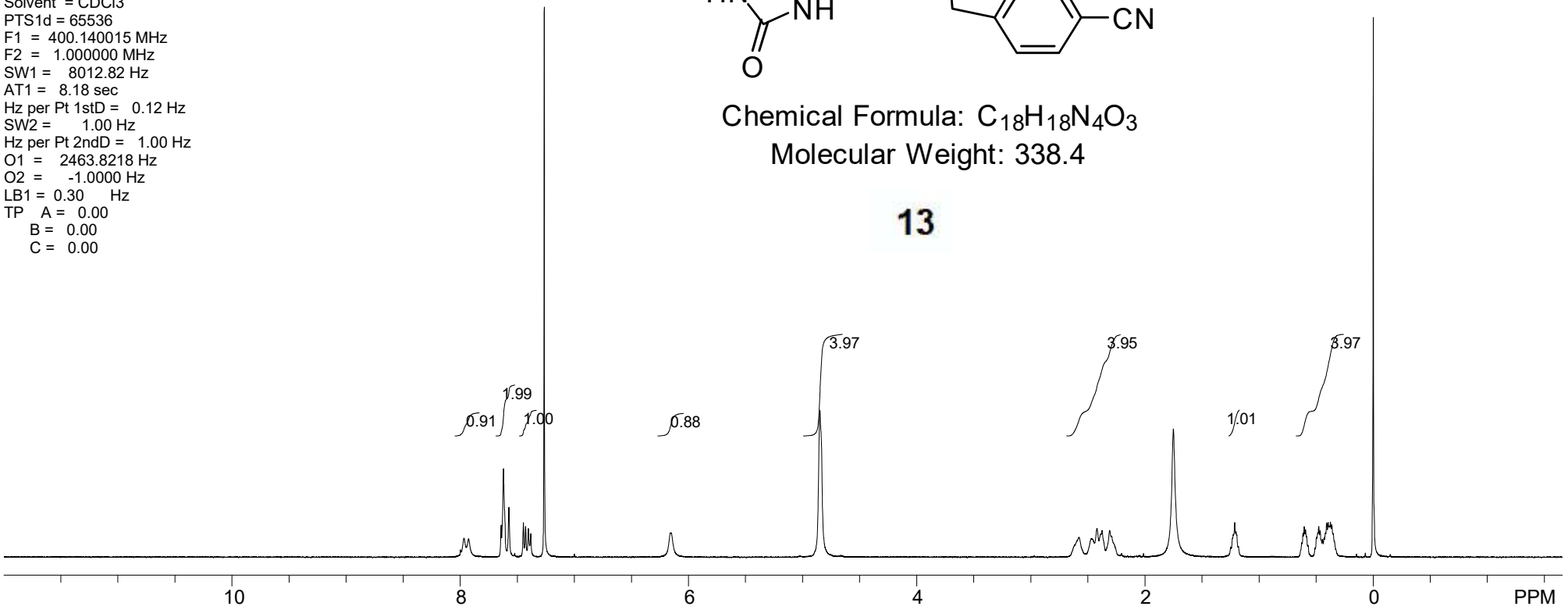
P:\PUBLIC\NMR_NUTS\New-nuts\nuts\DATA\$NMR-8075136-A1_1.1

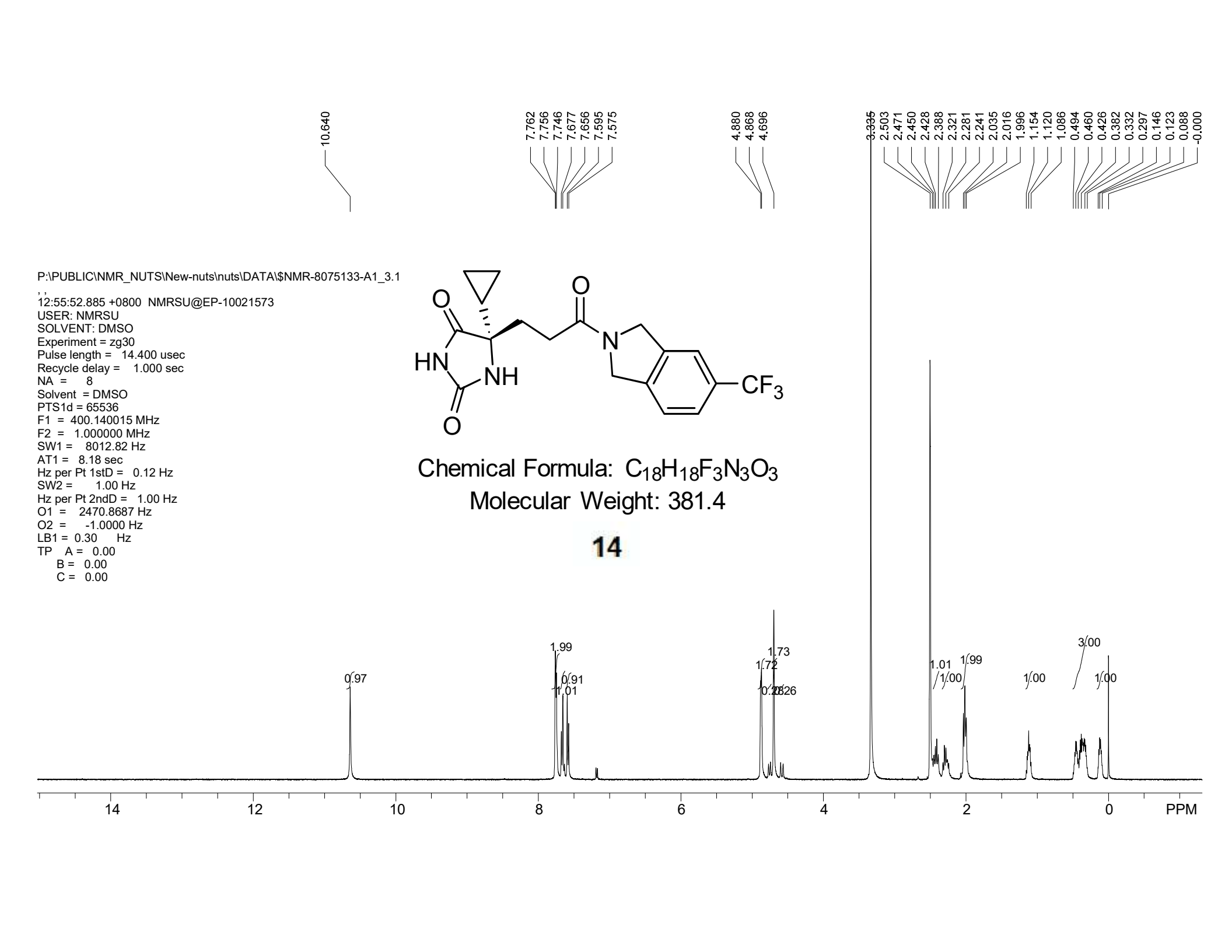
, ,
20:19:19.645 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: CDCl3
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 32
Solvent = CDCl3
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2463.8218 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00

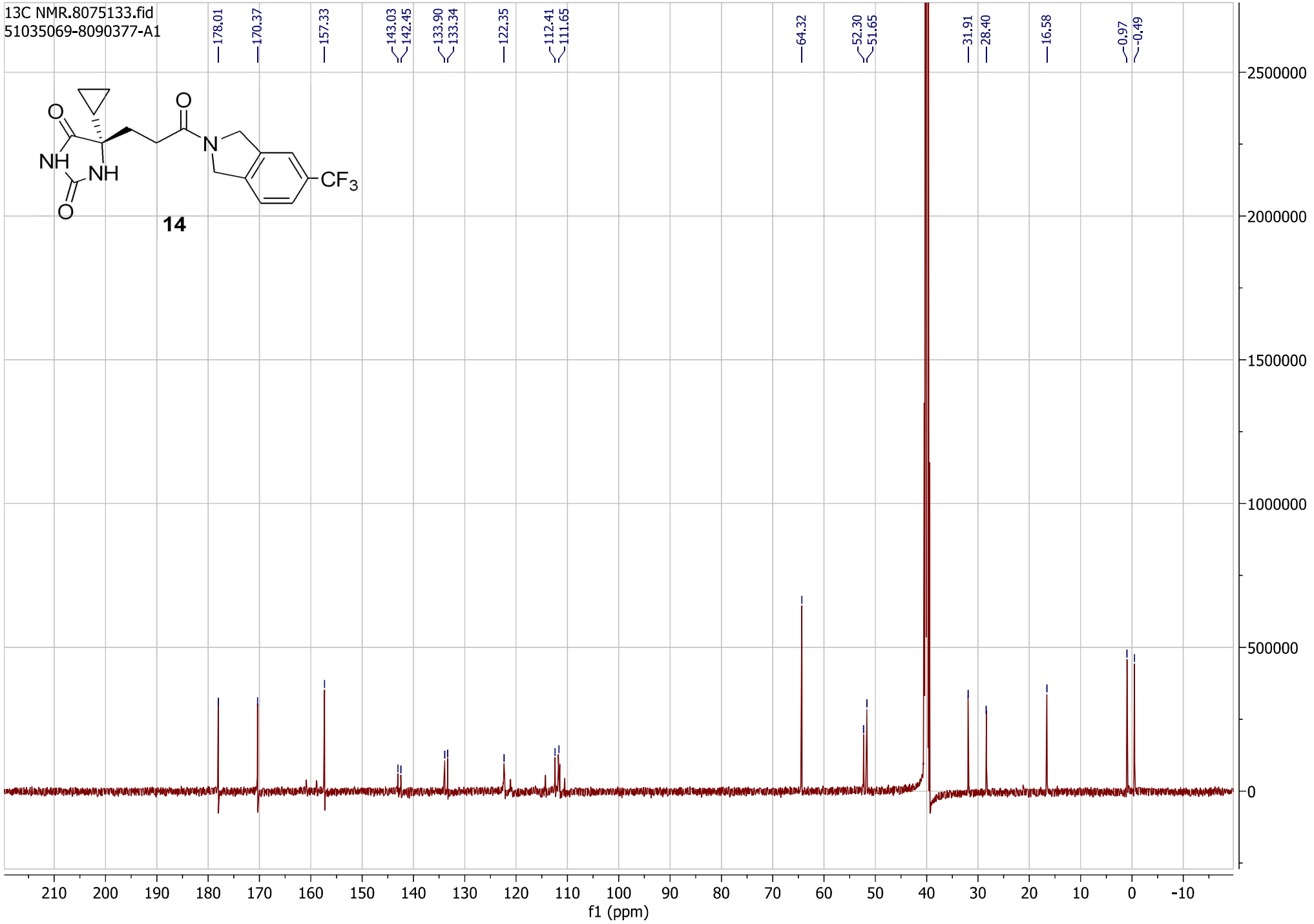


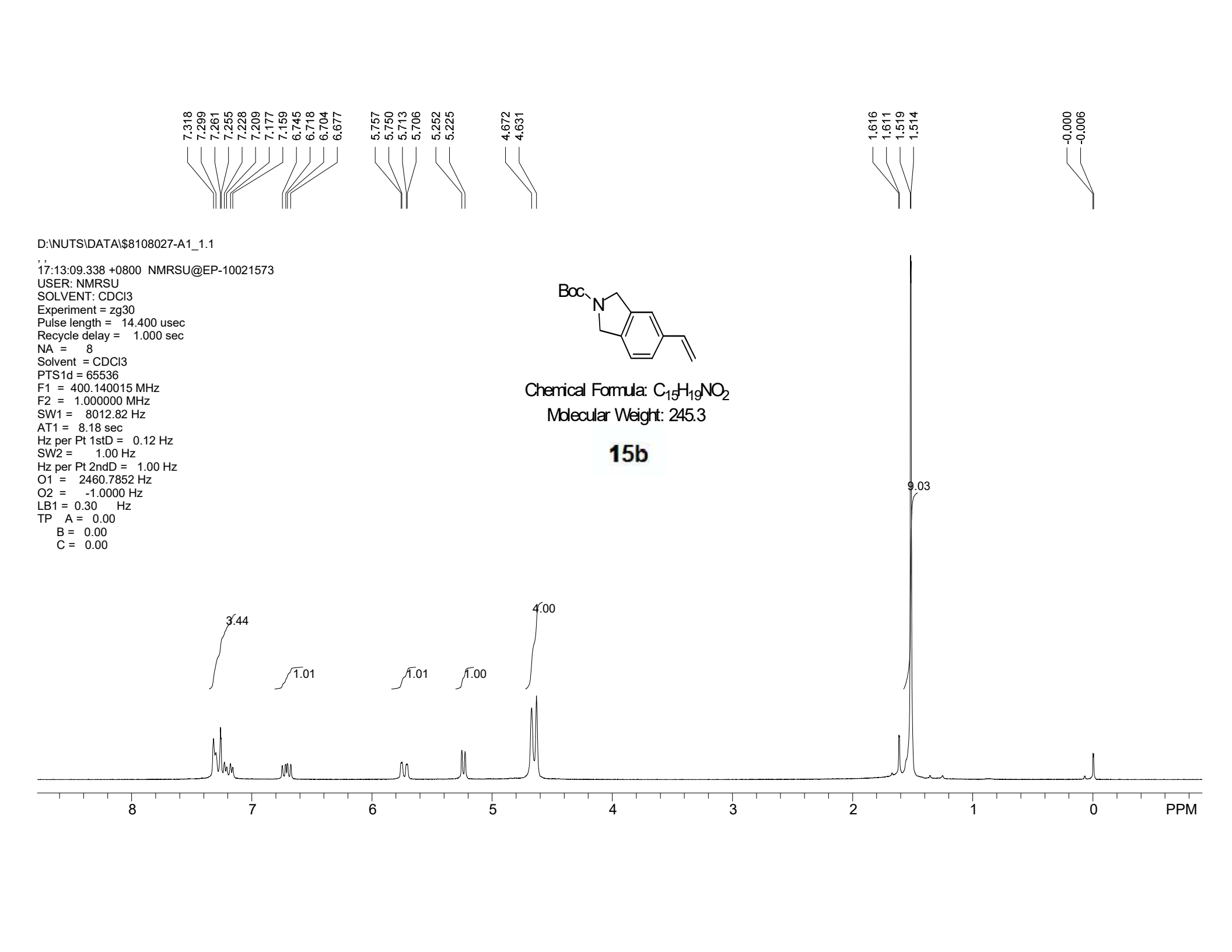
Chemical Formula: $C_{18}H_{18}N_4O_3$
Molecular Weight: 338.4

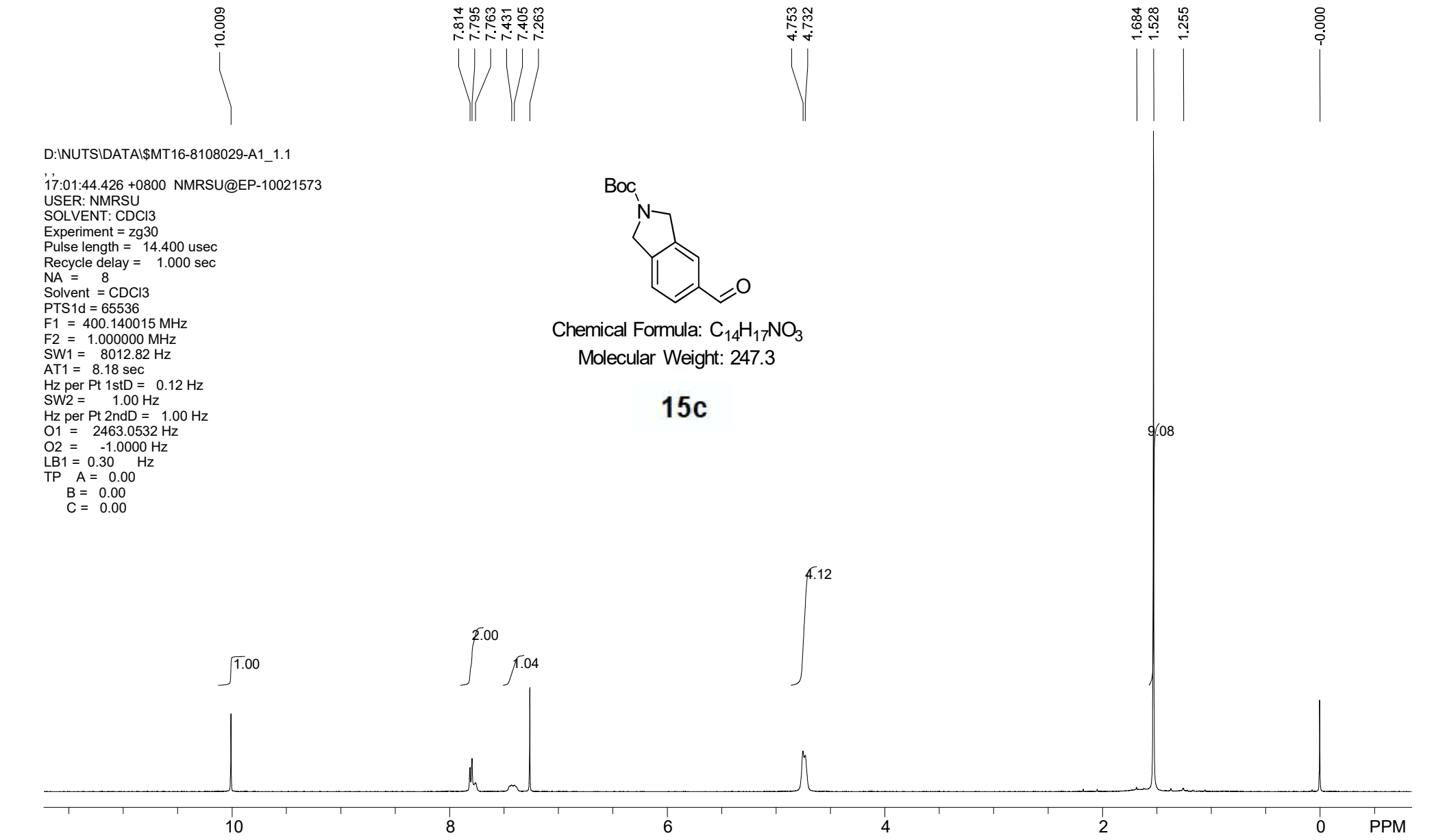
13

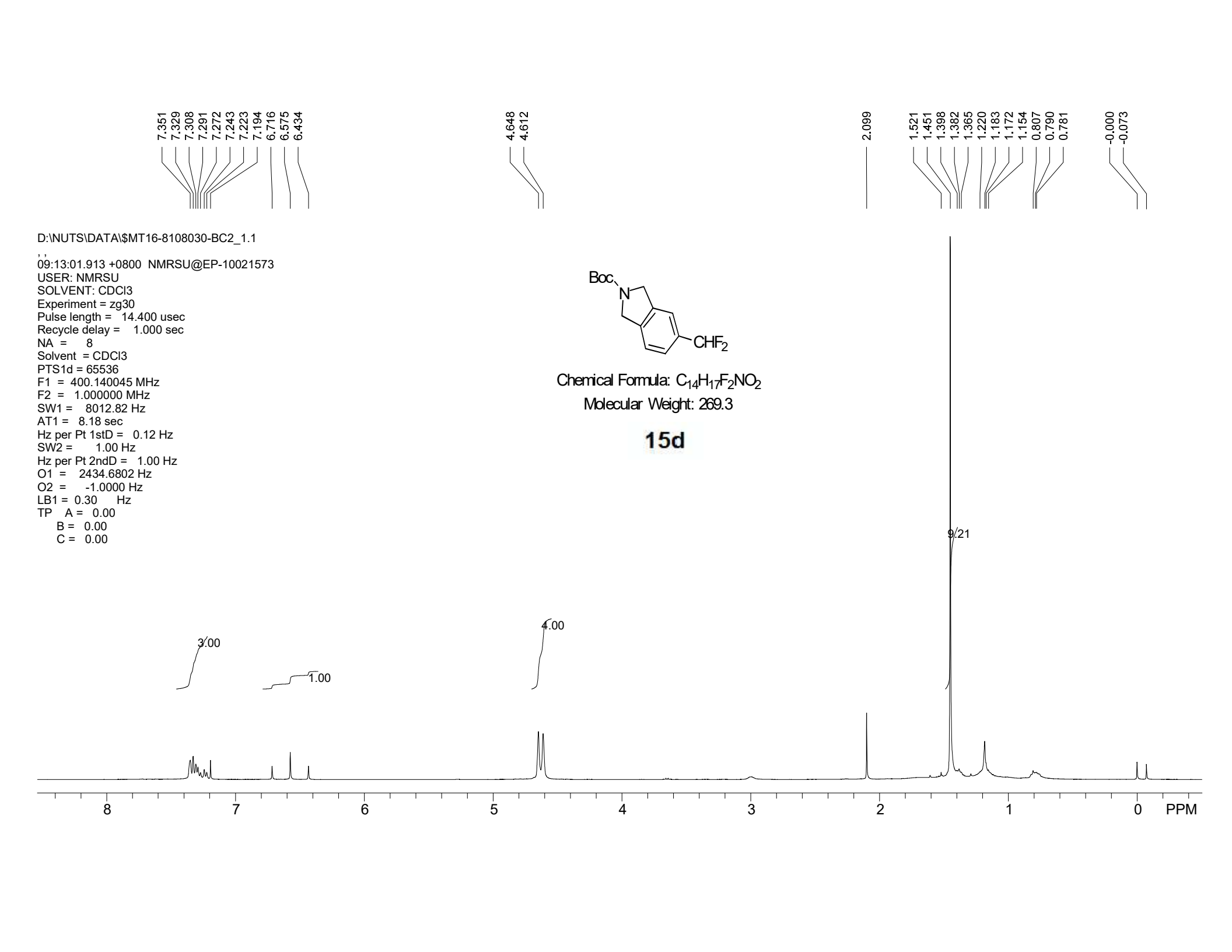


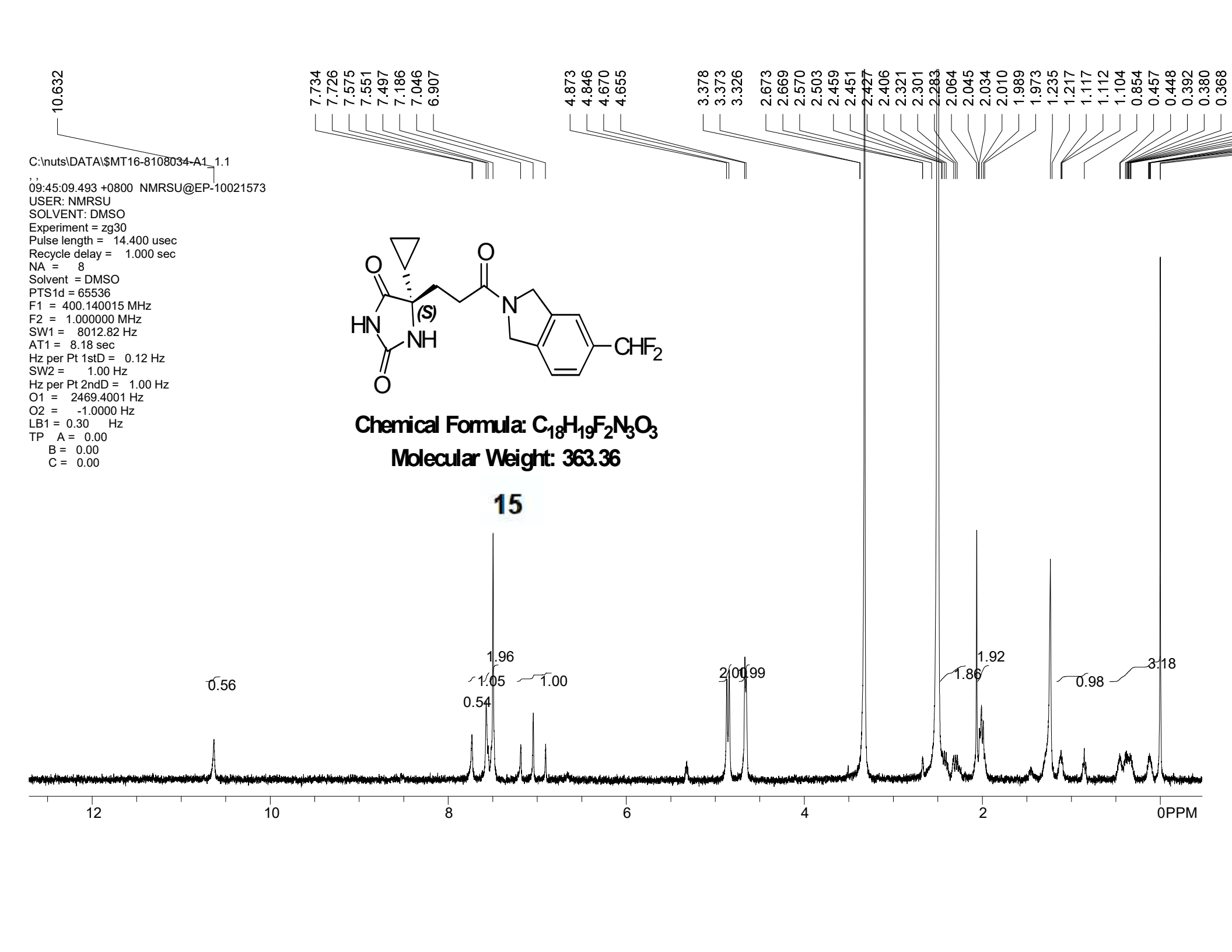

10.640
7.762
7.756
7.746
7.677
7.656
7.595
7.575
4.880
4.868
4.696
3.335
2.503
2.471
2.450
2.428
2.388
2.321
2.281
2.241
2.035
2.016
1.996
1.154
1.120
1.086
0.494
0.460
0.426
0.382
0.332
0.297
0.146
0.123
0.088
-0.000
P:\PUBLIC\NMR_NUTS\New-nuts\nuts\DATA\$NMR-8075133-A1_3.1
, ,
12:55:52.885 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2470.8687 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
O
HN
NH
O
O
N
CF3
Chemical Formula: C18H18F3N3O3
Molecular Weight: 381.4
14
0.97
1.99
0.91
1.01
1.73
1.72
0.28
0.26
1.01
1.00
1.99
1.00
3.00
1.00
14
12
10
8
6
4
2
0
PPM

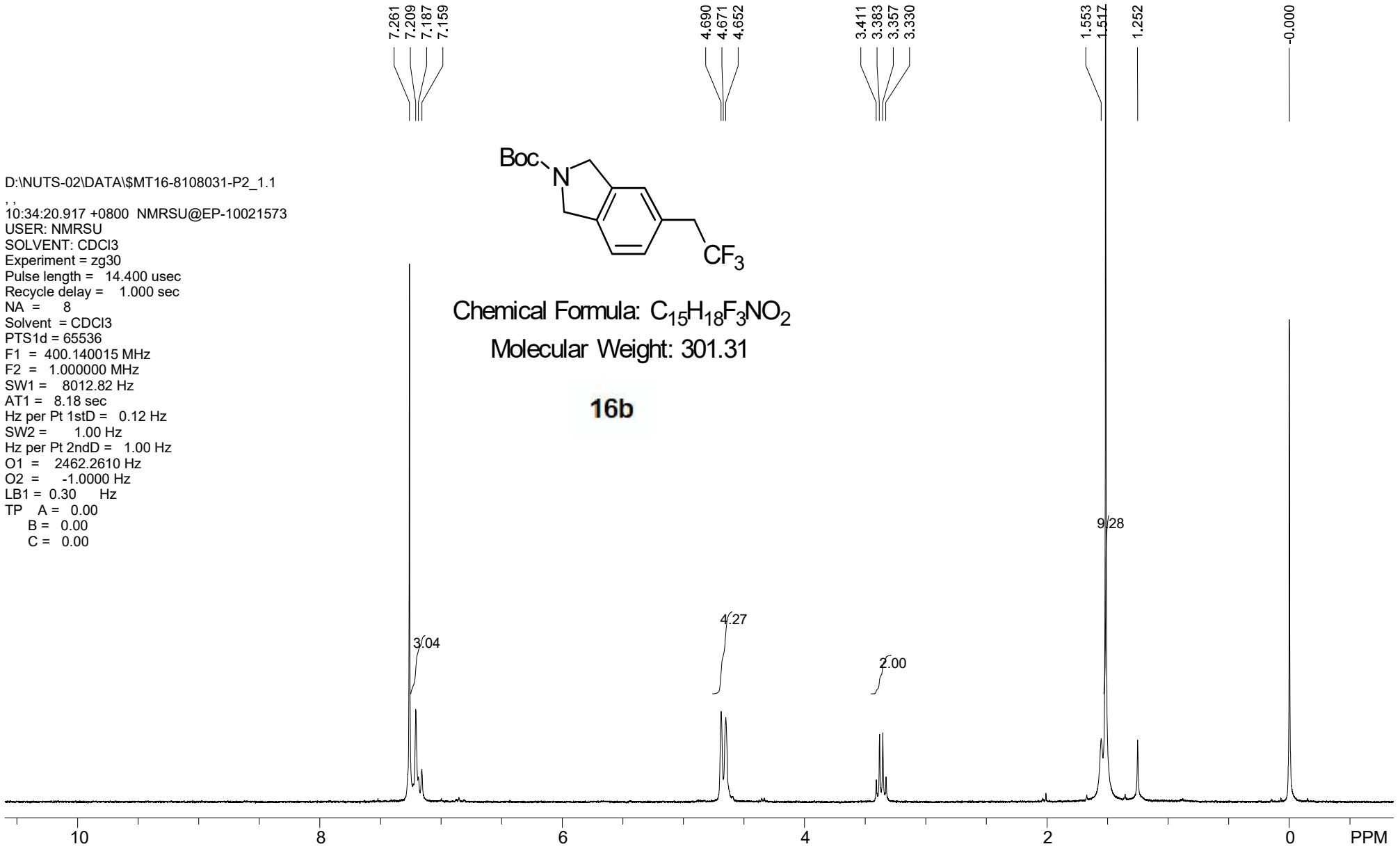

13C NMR.8075133.fid
51035069-8090377-A1
178.01
170.37
157.33
143.03
142.45
133.90
133.34
122.35
112.41
111.65
64.32
52.30
51.65
31.91
28.40
16.58
0.97
-0.49
O
NH
NH
O
O
N
CF3
14
2500000
2000000
1500000
1000000
500000
0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

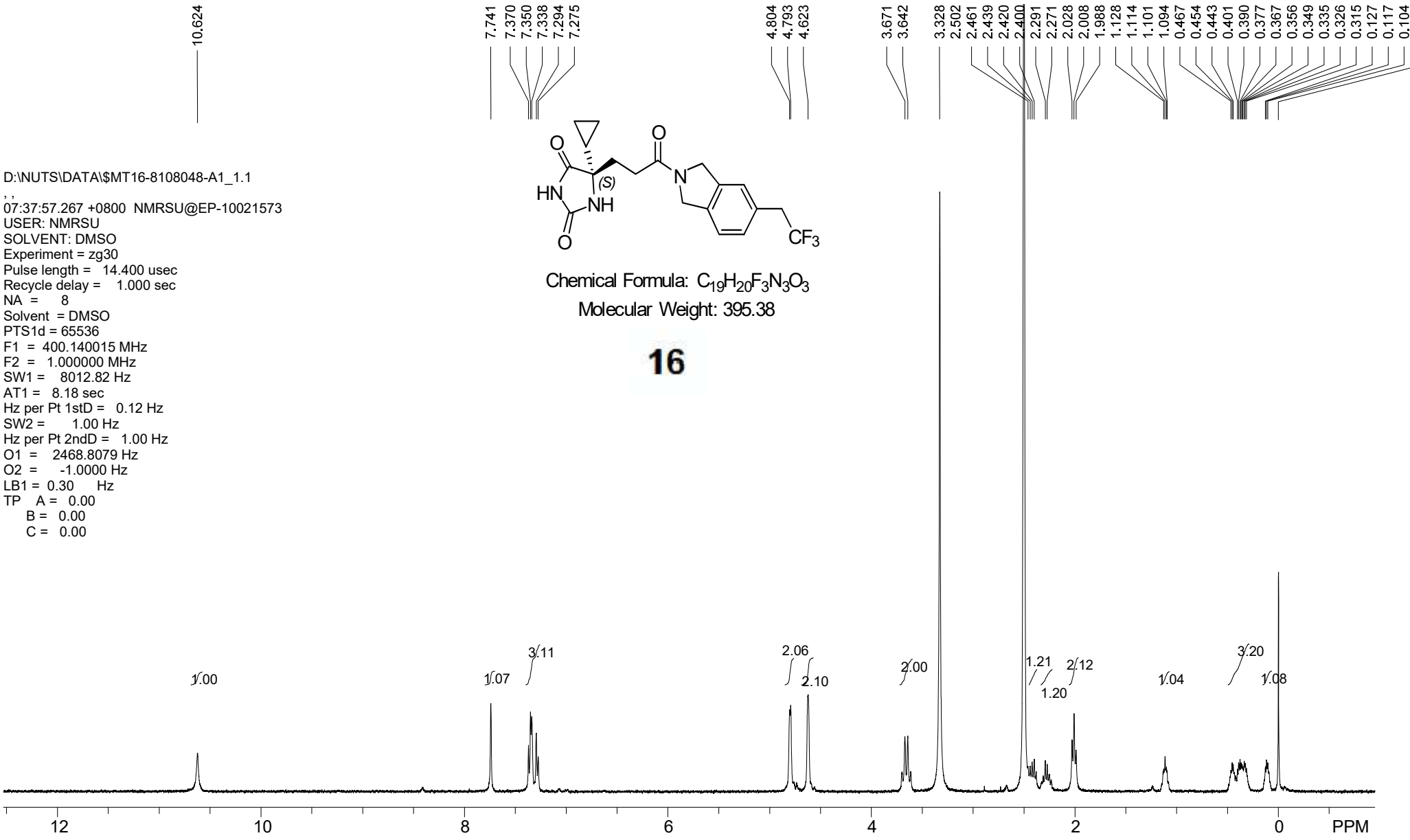

7.318
7.299
7.261
7.255
7.228
7.209
7.177
7.159
6.745
6.718
6.704
6.677
5.757
5.750
5.713
5.706
5.252
5.225
4.672
4.631
1.616
1.611
1.519
1.514
-0.000
-0.006
D:\NUTS\DATA\$8108027-A1_1.1
, ,
17:13:09.338 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: CDCl3
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = CDCl3
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2460.7852 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
Boc
N
Chemical Formula: C15H19NO2
Molecular Weight: 245.3
15b
3.44
1.01
1.01
1.00
4.00
9.03
8
7
6
5
4
3
2
1
0
PPM

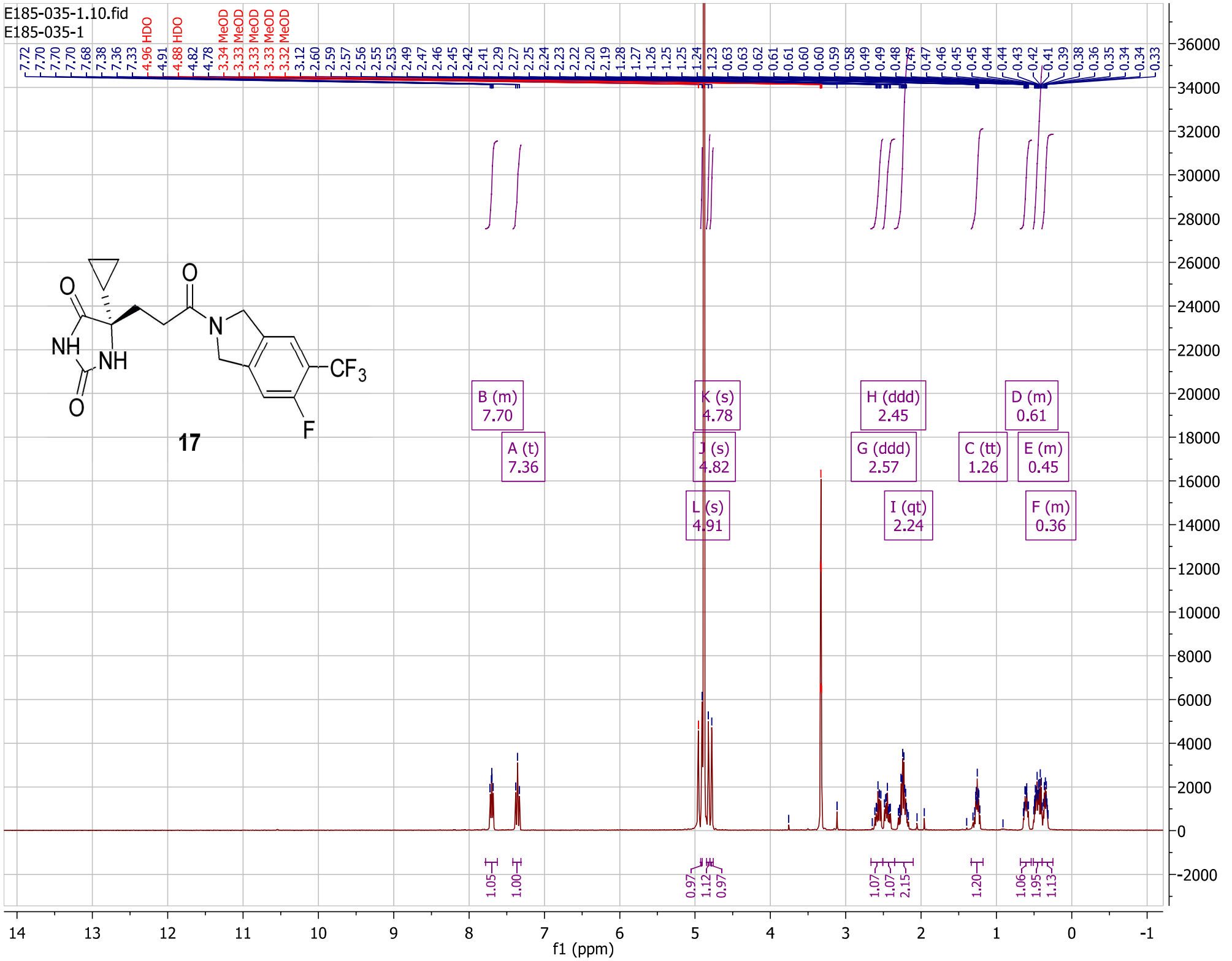

10.009
7.814
7.795
7.763
7.431
7.405
7.263
4.753
4.732
1.684
1.528
1.255
-0.000
D:\NUTS\DATA\$MT16-8108029-A1_1.1
, ,
17:01:44.426 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: CDCl3
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = CDCl3
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2463.0532 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
Boc
N
O
Chemical Formula: C14H17NO3
Molecular Weight: 247.3
15c
9.08
4.12
2.00
1.04
1.00
10
8
6
4
2
0
PPM

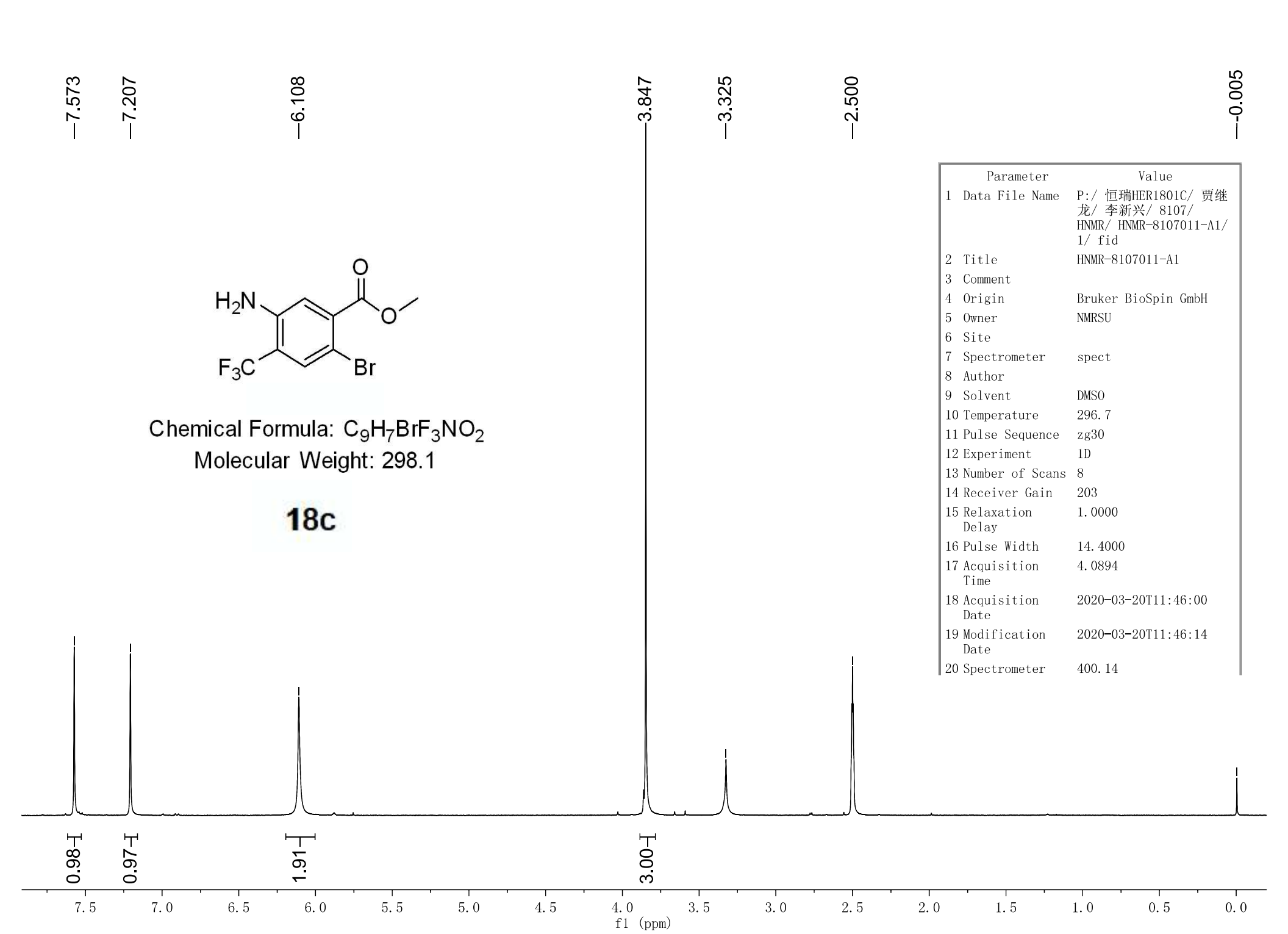

7.351
7.329
7.308
7.291
7.272
7.243
7.223
7.194
6.716
6.575
6.434
4.648
4.612
2.099
1.521
1.451
1.398
1.382
1.365
1.220
1.183
1.172
1.154
0.807
0.790
0.781
-0.000
-0.073
D:\NUTS\DATA\$MT16-8108030-BC2_1.1
, ,
09:13:01.913 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: CDCl3
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = CDCl3
PTS1d = 65536
F1 = 400.140045 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2434.6802 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
Boc
N
CHF2
Chemical Formula: C14H17F2NO2
Molecular Weight: 269.3
15d
9.21
4.00
3.00
1.00
8
7
6
5
4
3
2
1
0
PPM


10.632
7.734
7.726
7.575
7.551
7.497
7.186
7.046
6.907
4.873
4.846
4.670
4.655
3.378
3.373
3.326
2.673
2.669
2.570
2.503
2.459
2.451
2.427
2.406
2.321
2.301
2.283
2.064
2.045
2.034
2.010
1.989
1.973
1.235
1.217
1.117
1.112
1.104
0.854
0.457
0.448
0.392
0.380
0.368
C:\nuts\DATA\$MT16-8108034-A1_1.1
, ,
09:45:09.493 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2469.4001 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
O
O
HN
NH
O
(S)
N
CHF2
Chemical Formula: C18H19F2N3O3
Molecular Weight: 363.36
15
0.56
1.96
1.05
0.54
1.00
2.01
0.99
1.86
1.92
0.98
3.18
12
10
8
6
4
2
0PPM


7.261
7.209
7.187
7.159
4.690
4.671
4.652
3.411
3.383
3.357
3.330
1.553
1.517
1.252
-0.000
Boc
N
CF3
Chemical Formula: C15H18F3NO2
Molecular Weight: 301.31
16b
D:\NUTS-02\DATA\$MT16-8108031-P2_1.1
, ,
10:34:20.917 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: CDCl3
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = CDCl3
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2462.2610 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
3.04
4.27
2.00
9.28
10
8
6
4
2
0
PPM


10.624
7.741
7.370
7.350
7.338
7.294
7.275
4.804
4.793
4.623
3.671
3.642
3.328
2.502
2.461
2.439
2.420
2.400
2.291
2.271
2.028
2.008
1.988
1.128
1.114
1.101
1.094
0.467
0.454
0.443
0.401
0.390
0.377
0.367
0.356
0.349
0.335
0.326
0.315
0.127
0.117
0.104
D:\NUTS\DATA\$MT16-8108048-A1_1.1
, ,
07:37:57.267 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2468.8079 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
O
O
HN
NH
(S)
N
O
CF3
Chemical Formula: C19H20F3N3O3
Molecular Weight: 395.38
16
1.00
1.07
3.11
2.06
2.10
2.00
1.21
1.20
2.12
1.04
3.20
1.08
12
10
8
6
4
2
0
PPM

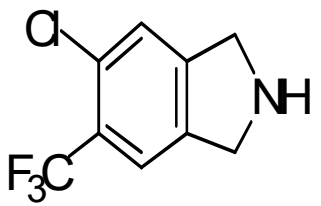

E185-035-1.10.fid
E185-035-1
HDO
MeOD
B (m) 7.70
A (t) 7.36
K (s) 4.78
J (s) 4.82
L (s) 4.91
H (ddd) 2.45
G (ddd) 2.57
I (qt) 2.24
C (tt) 1.26
D (m) 0.61
E (m) 0.45
F (m) 0.36
17
f1 (ppm)



	Parameter	Value
1	Data File Name	P:/ 恒瑞HER1801C/ 贾继龙/ 李新兴/ 8107/ HNMR/ HNMR-8107011-A1/ 1/ fid
2	Title	HNMR-8107011-A1
3	Comment	
4	Origin	Bruker BioSpin GmbH
5	Owner	NMRSU
6	Site	
7	Spectrometer	spect
8	Author	
9	Solvent	DMSO
10	Temperature	296.7
11	Pulse Sequence	zg30
12	Experiment	1D
13	Number of Scans	8
14	Receiver Gain	203
15	Relaxation Delay	1.0000
16	Pulse Width	14.4000
17	Acquisition Time	4.0894
18	Acquisition Date	2020-03-20T11:46:00
19	Modification Date	2020-03-20T11:46:14
20	Spectrometer	400.14

7.780
7.648

4.158
4.136

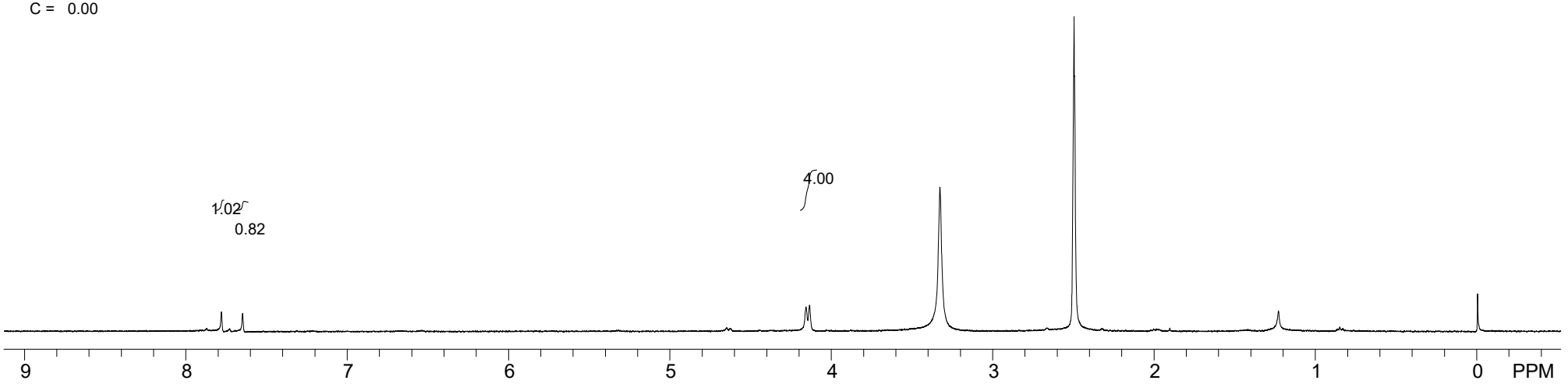


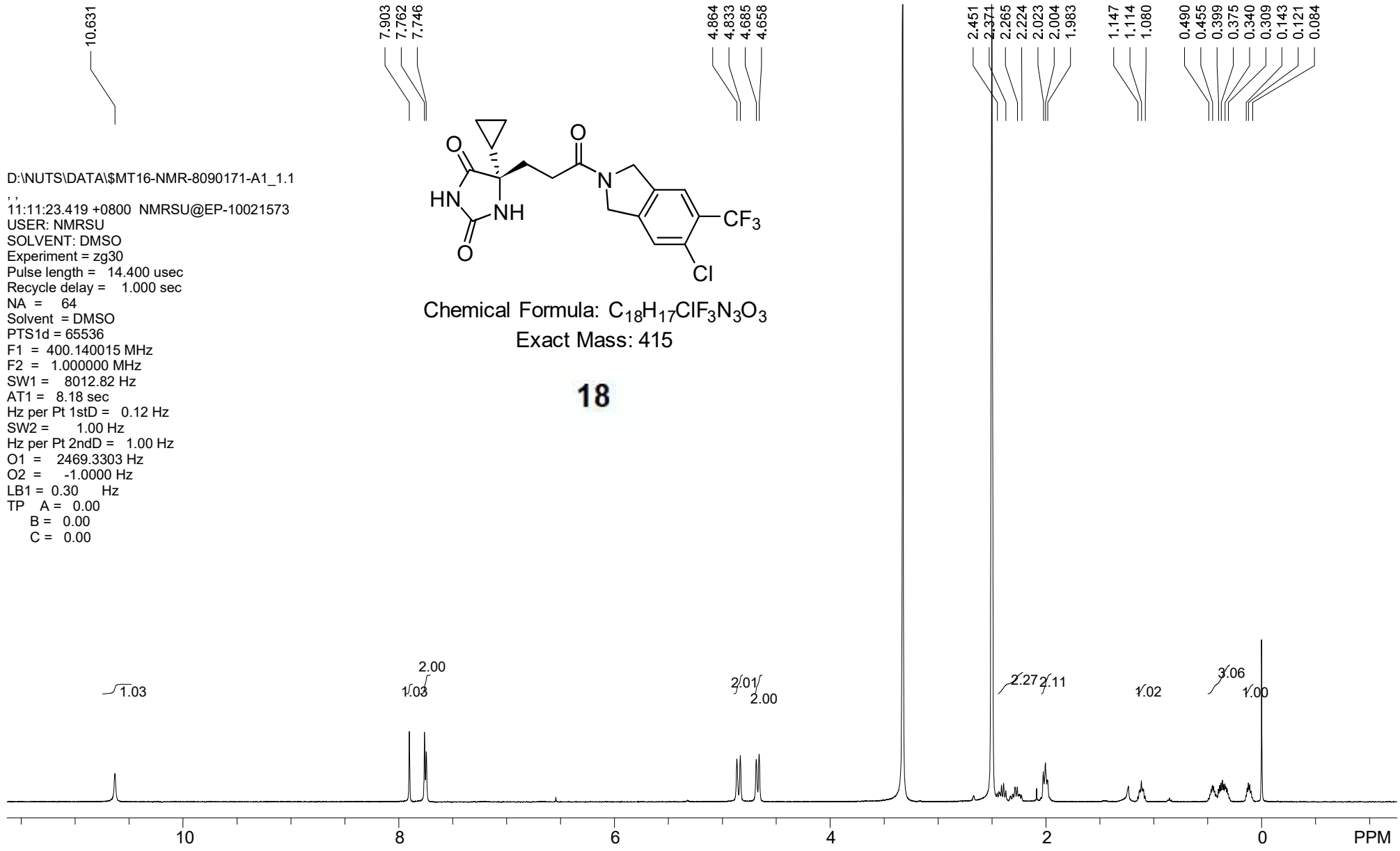
D:\NUTS\DATA\$MT16-NMR-8090167-A1_1.1
, ,
10:55:44.020 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2467.3740 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00

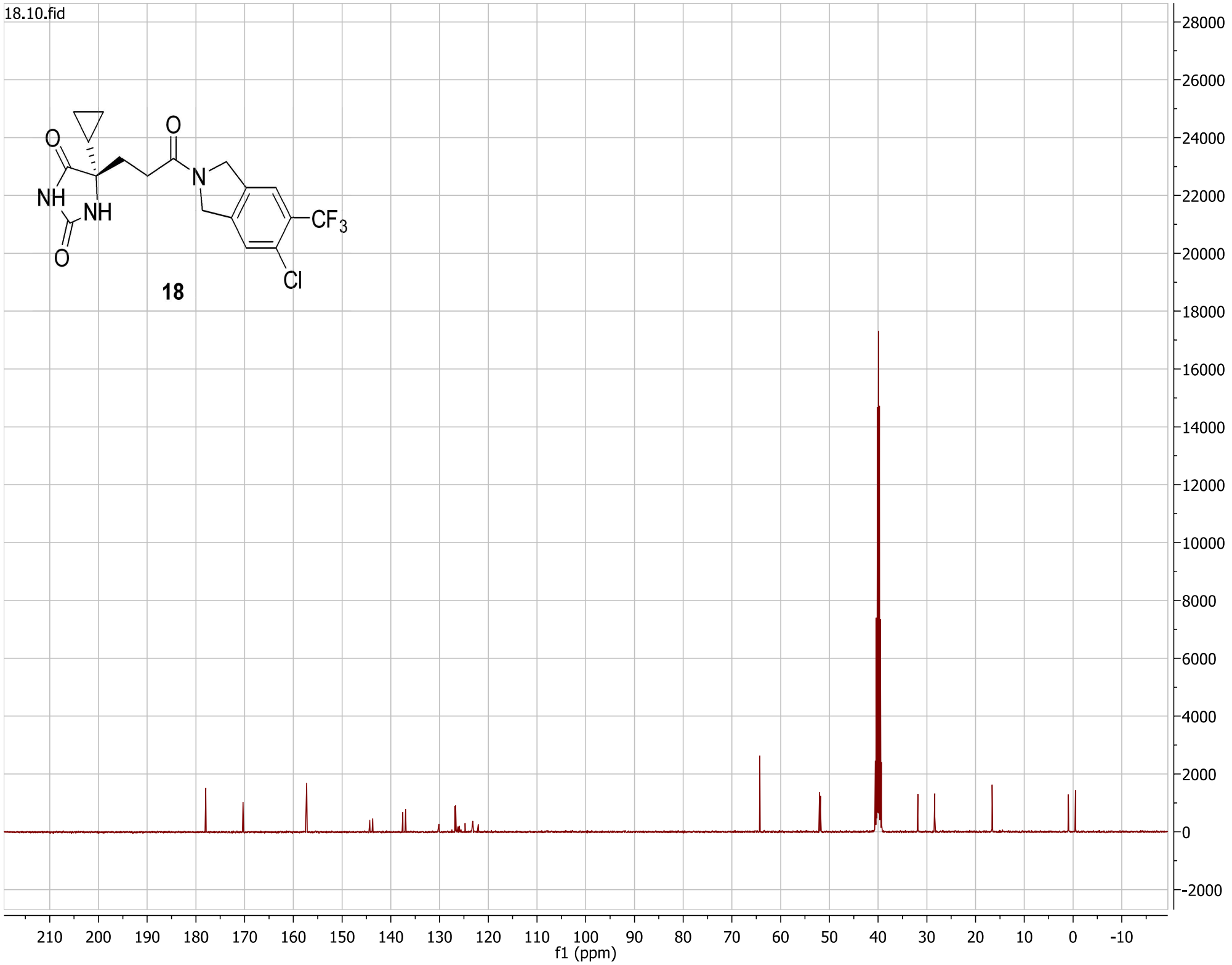
Chemical Formula: $C_9H_7ClF_3N$

Molecular Weight: 222

18h




10.631
7.903
7.762
7.746
4.864
4.833
4.685
4.658
2.451
2.371
2.265
2.224
2.023
2.004
1.983
1.147
1.114
1.080
0.490
0.455
0.399
0.375
0.340
0.309
0.143
0.121
0.084
D:\NUTS\DATA\$MT16-NMR-8090171-A1_1.1
, ,
11:11:23.419 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 64
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2469.3303 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
O
O
HN
NH
O
N
CF3
Cl
Chemical Formula: C18H17ClF3N3O3
Exact Mass: 415
18
1.03
2.00
1.03
2.01
2.00
2.27
2.11
1.02
3.06
1.00
10
8
6
4
2
0
PPM


18.10.fid
O
O
NH
NH
O
N
CF3
Cl
18
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

18 HPLC

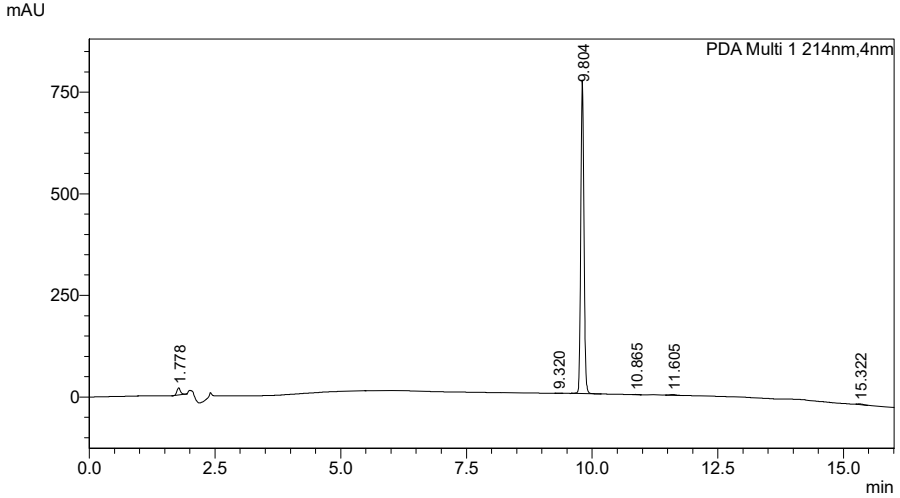
HPLC Report

<Sample Information>

Sample Name	: MT16-HPLC-8090171-A1	Method Filename	: M-A80B20.lcm
Data Filename	: MT16-HPLC-8090171-A1-LC001.lcd	Vial #	: 1-20
Batch Filename	: 20200423.lcb	Injection Volume	: 20 uL
Date Acquired	: 4/23/2020 2:26:45 PM	Acquired by	: System Administrator
Date Processed	: 4/23/2020 2:43:35 PM	Processed by	: System Administrator

SunFire C18 5um 4.6x150mm 25C 1.000mlmin 16min
<<Mobile Phase Name>>
Pump A Mobile Phase A : 0.03%TFA in H2O
Pump B Mobile Phase A : 0.03%TFA in ACN

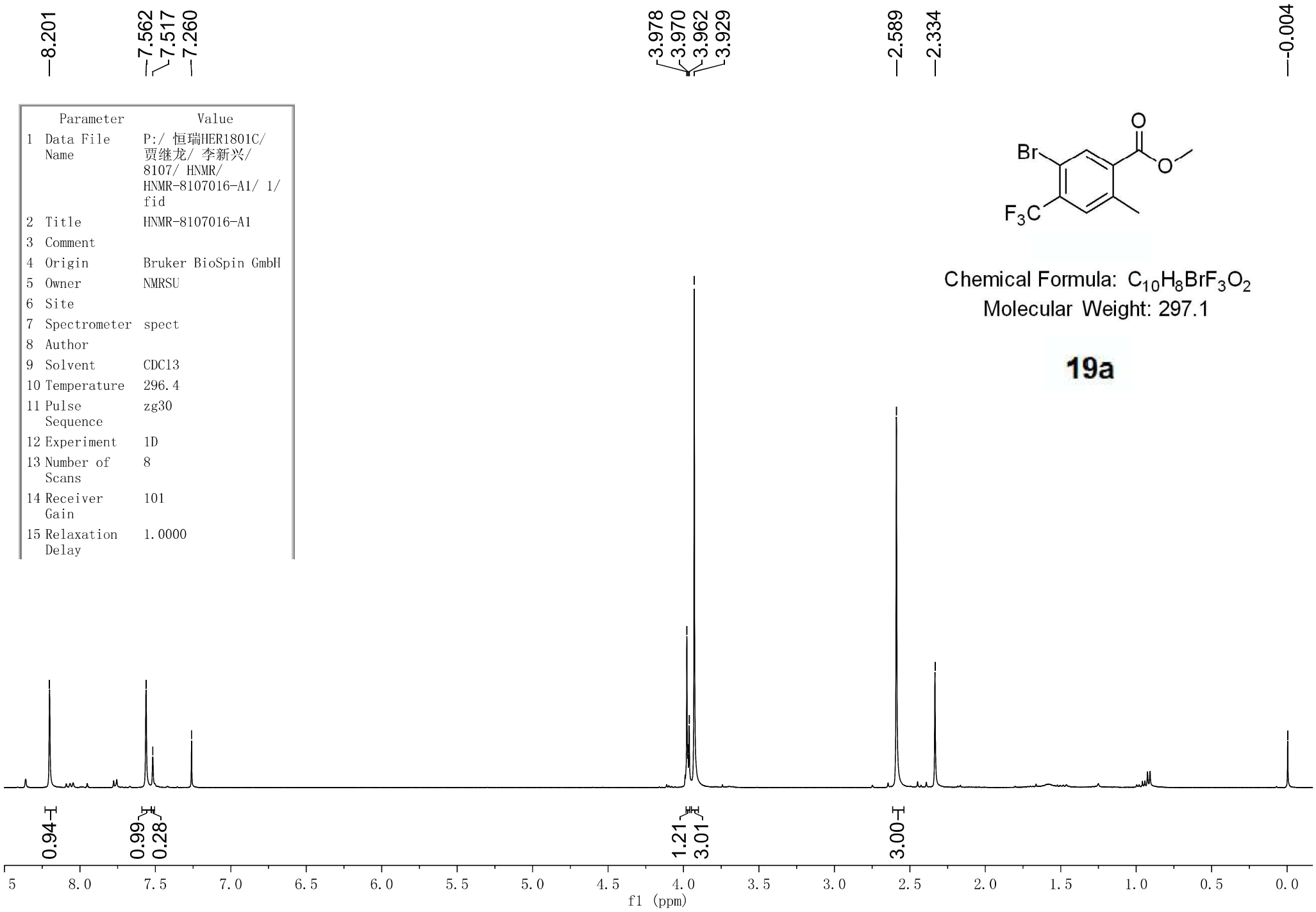
<Chromatogram>

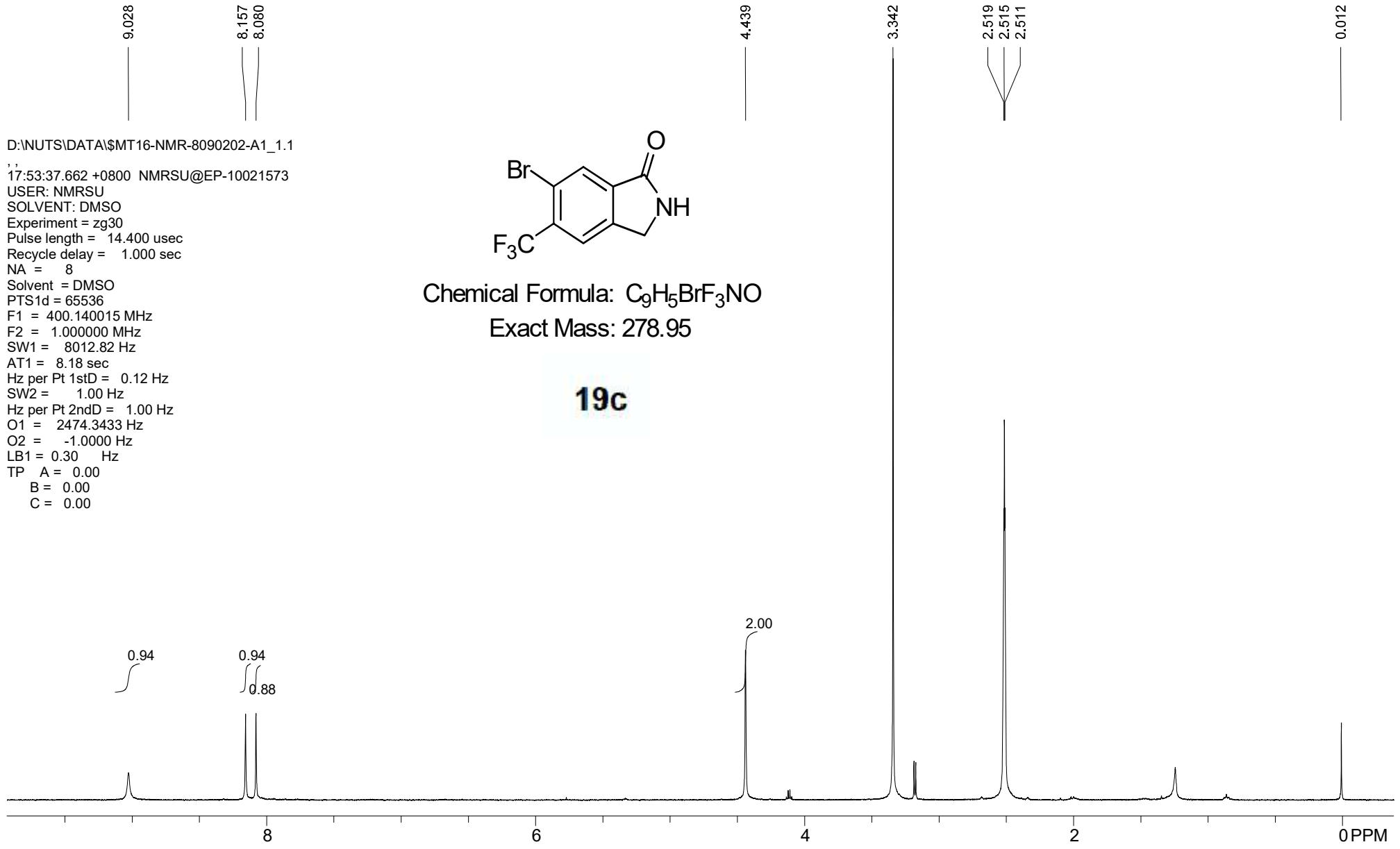


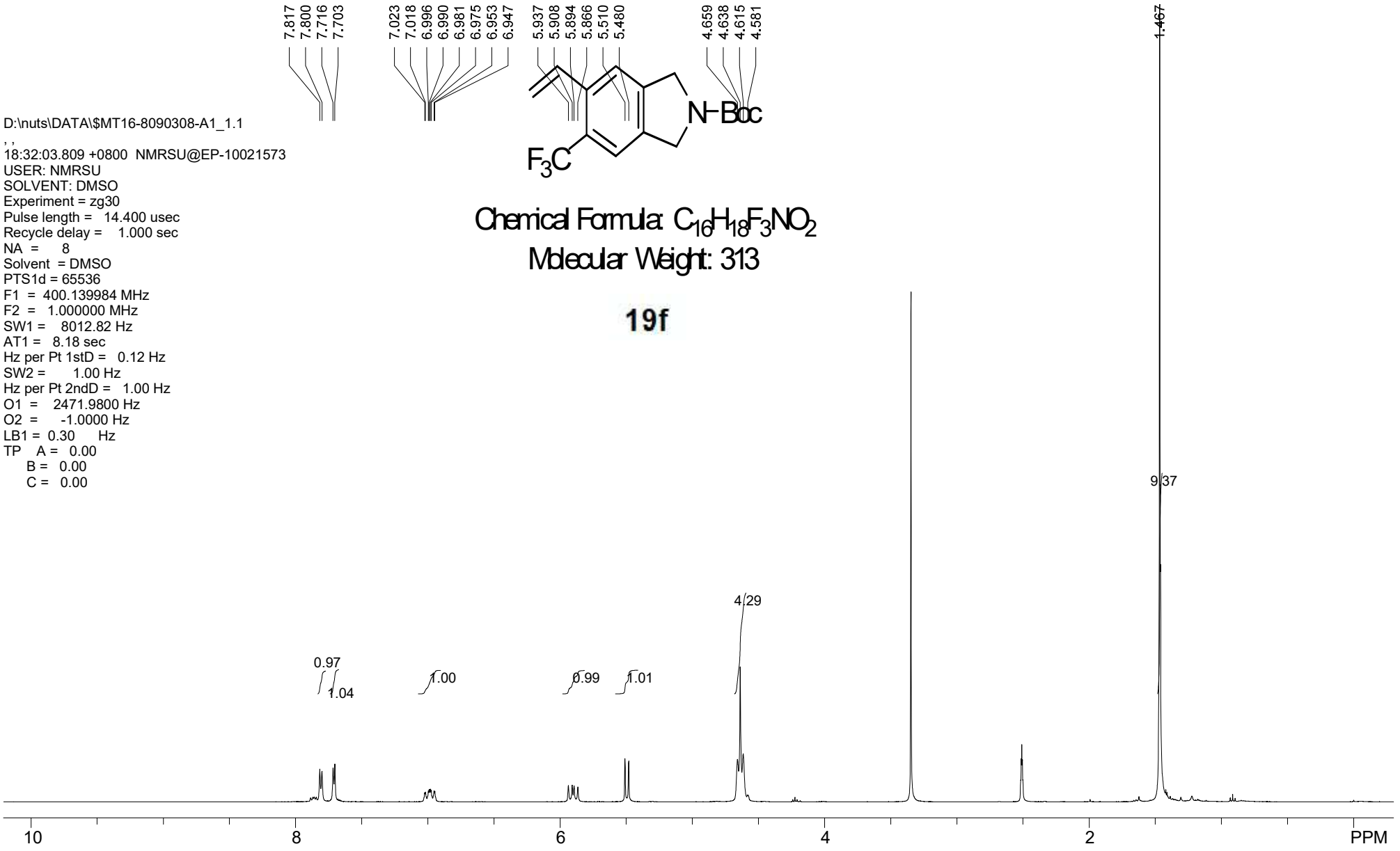
<Peak Table>

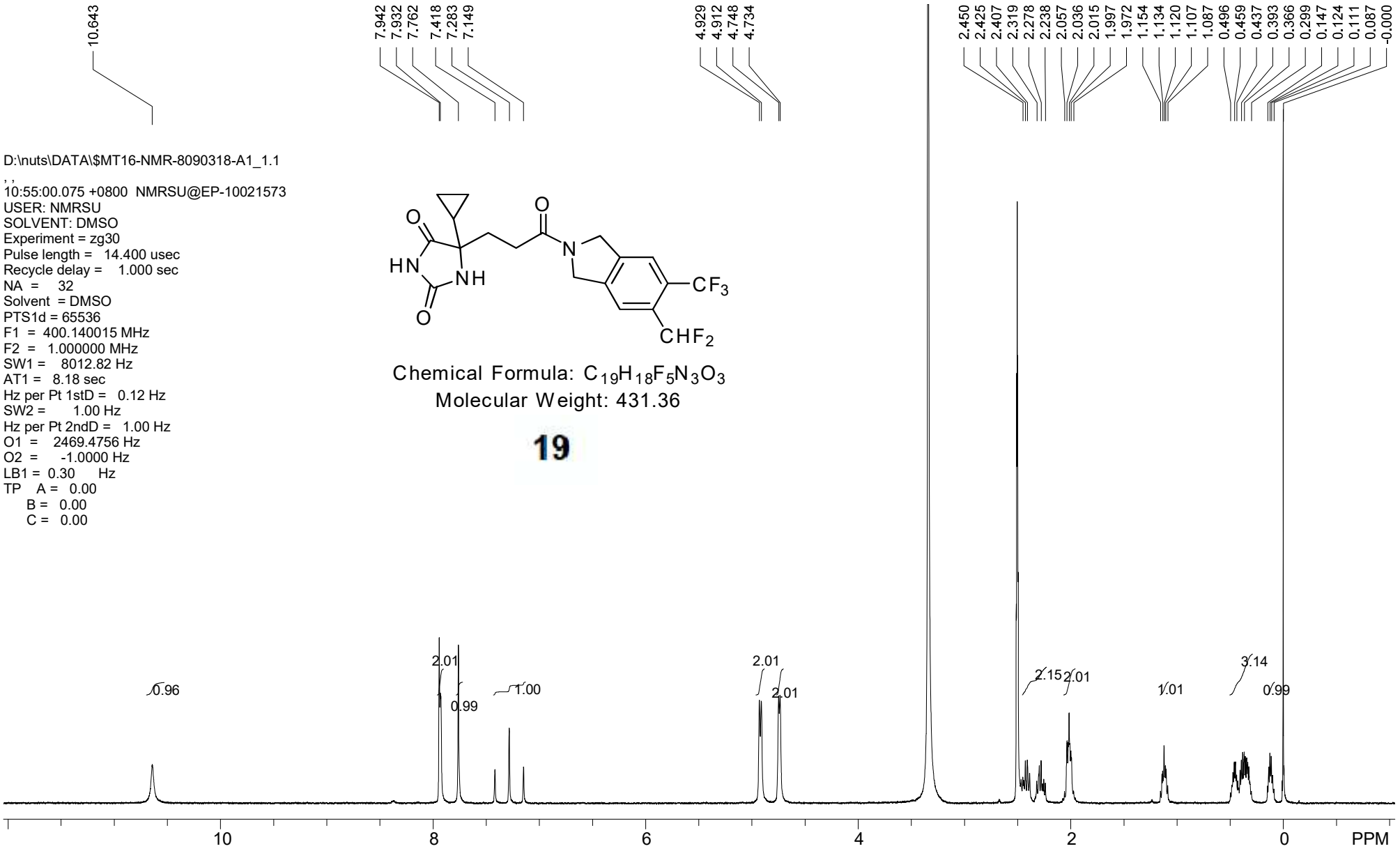
PDA Ch1 214nm

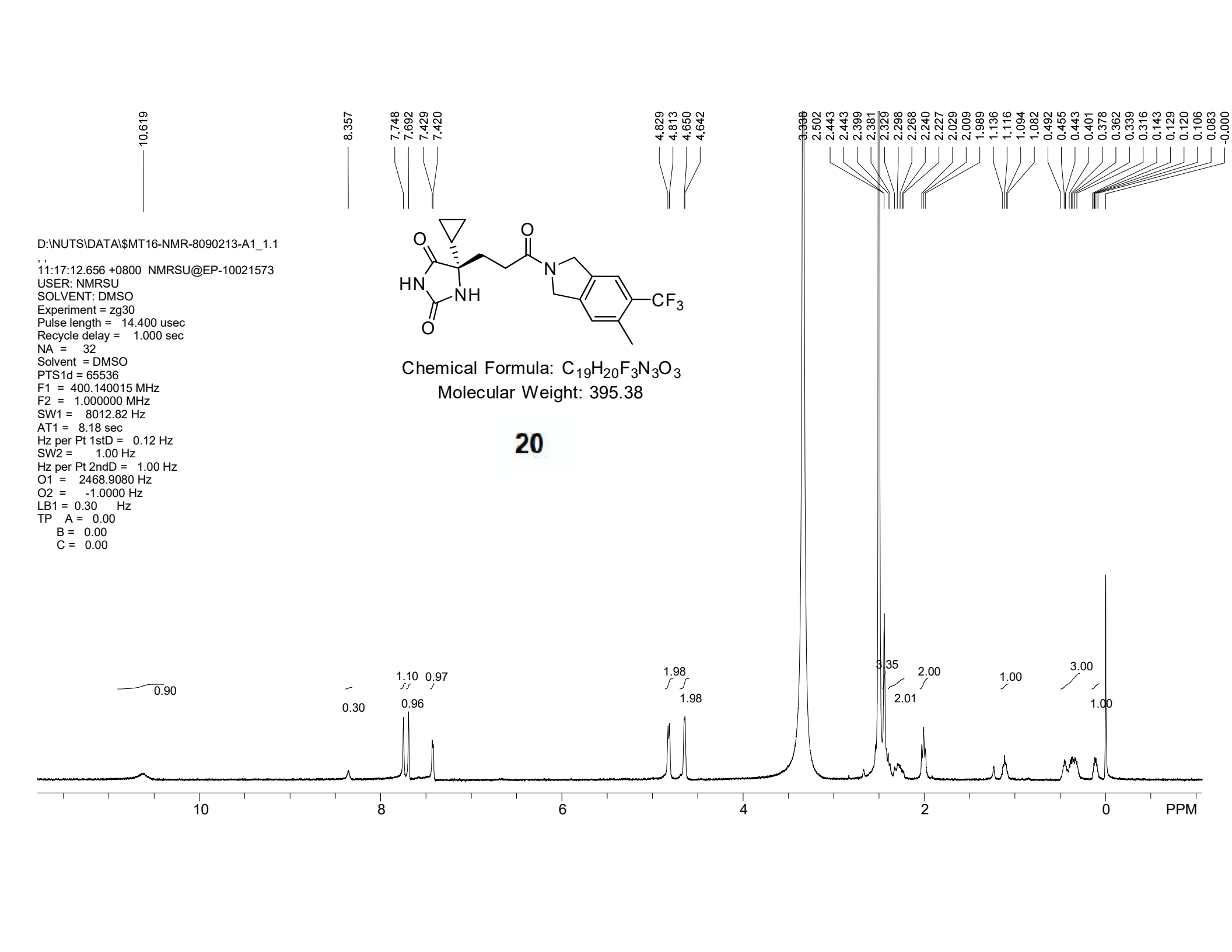
Peak#	Ret. Time	USP Width	Height	Area	S/N	Area%
1	1.778	0.138	17784	89182	10.74	2.569
2	9.320	0.119	427	1656	0.26	0.048
3	9.804	0.121	771437	3353118	465.90	96.597
4	10.865	0.126	608	2564	0.37	0.074
5	11.605	0.227	1793	14576	1.08	0.420
6	15.322	0.184	1413	10162	0.85	0.293
Total				3471258		100.000

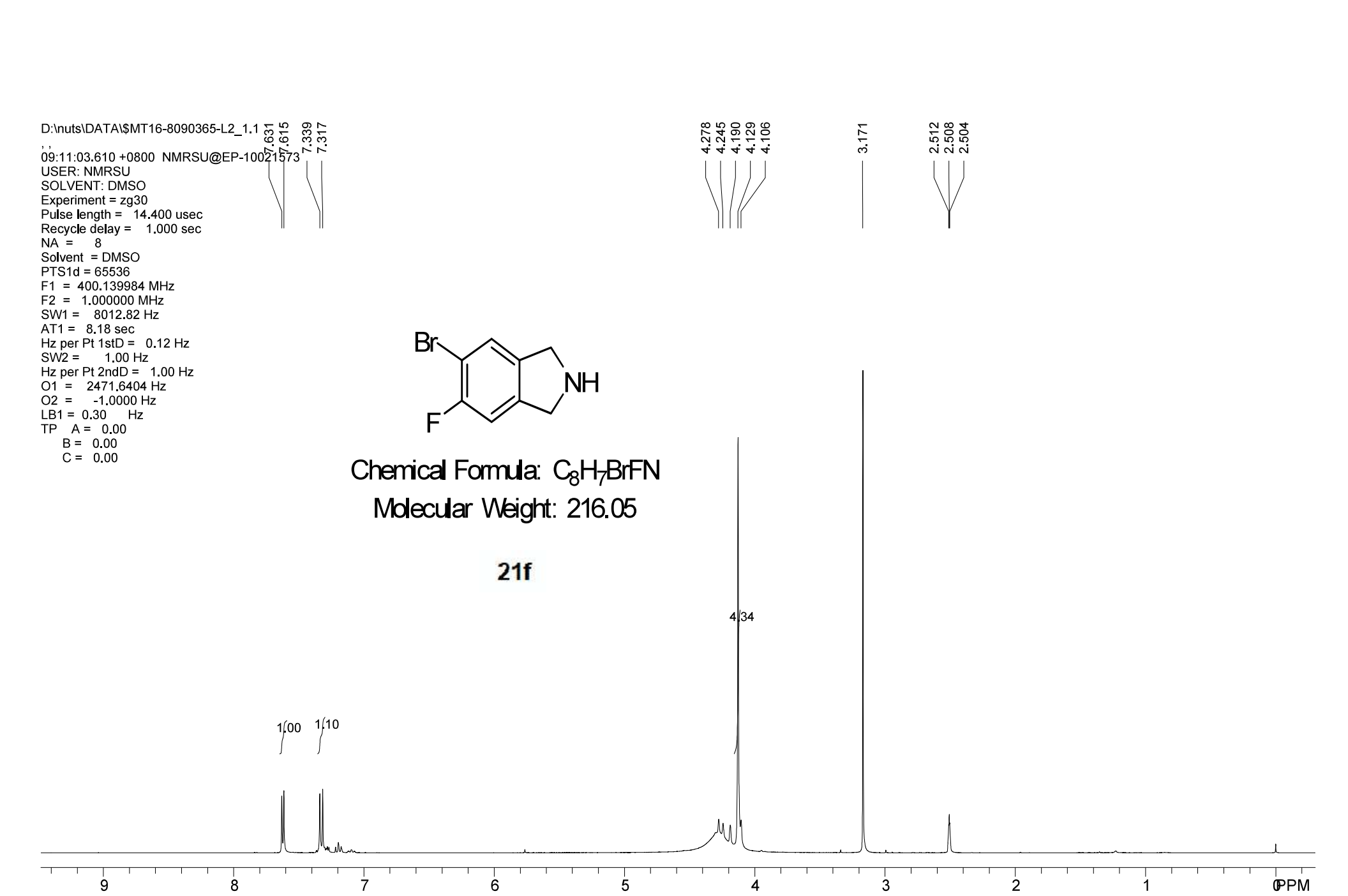

8.201
7.562
7.517
7.260
3.978
3.970
3.962
3.929
2.589
2.334
0.004
Parameter Value
1 Data File Name P:/ 恒瑞HER1801C/ 贾继龙/ 李新兴/ 8107/ HNMR/ HNMR-8107016-A1/ 1/ fid
2 Title HNMR-8107016-A1
3 Comment
4 Origin Bruker BioSpin GmbH
5 Owner NMRSU
6 Site
7 Spectrometer spect
8 Author
9 Solvent CDCl3
10 Temperature 296.4
11 Pulse Sequence zg30
12 Experiment 1D
13 Number of Scans 8
14 Receiver Gain 101
15 Relaxation Delay 1.0000
Br
O
O
F3C
Chemical Formula: C10H8BrF3O2
Molecular Weight: 297.1
19a
0.94
0.99
0.28
1.21
3.01
3.00
5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

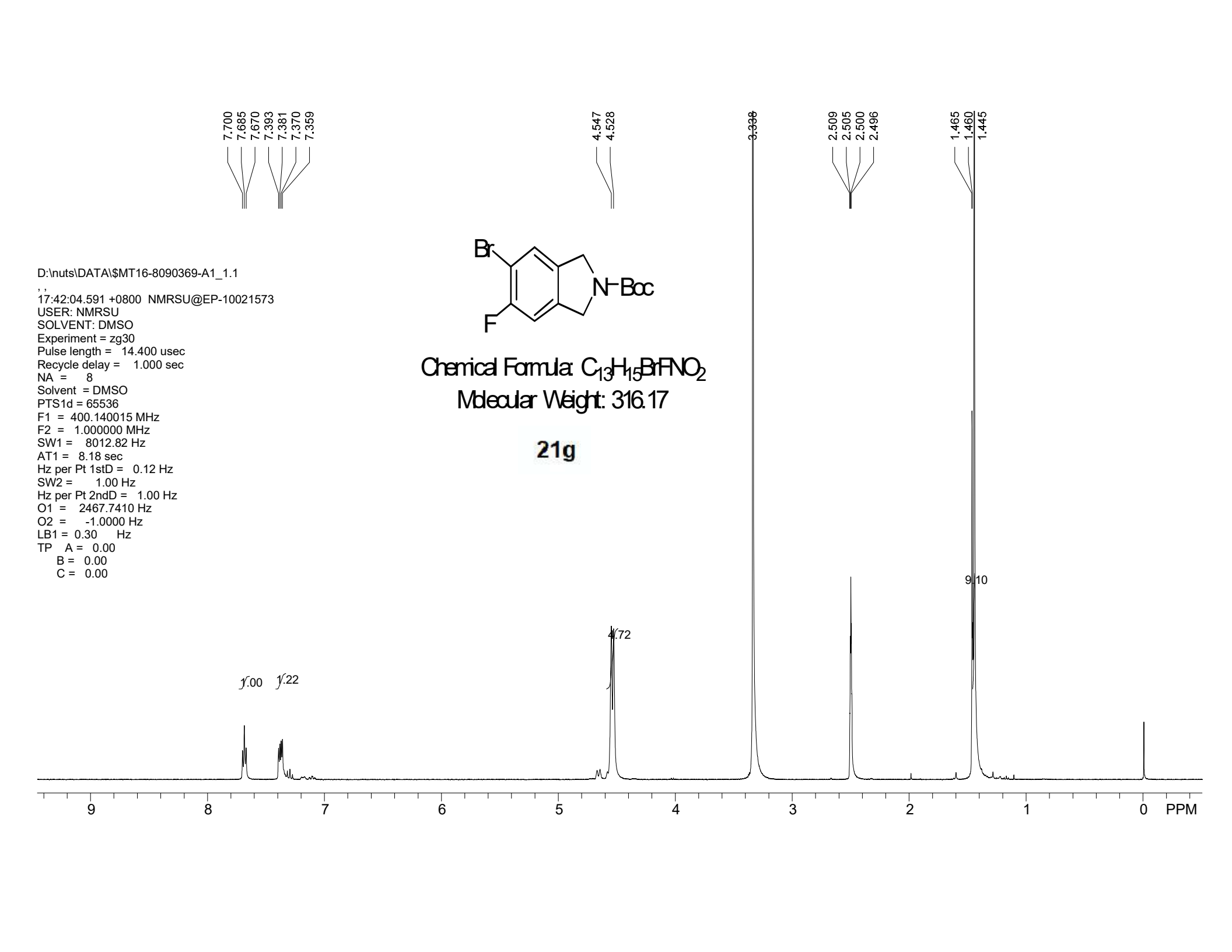

9.028
8.157
8.080
4.439
3.342
2.519
2.515
2.511
0.012
D:\NUTS\DATA\$MT16-NMR-8090202-A1_1.1
, ,
17:53:37.662 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2474.3433 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
Br
O
NH
F3C
Chemical Formula: C9H5BrF3NO
Exact Mass: 278.95
19c
0.94
0.94
0.88
2.00
8
6
4
2
0 PPM

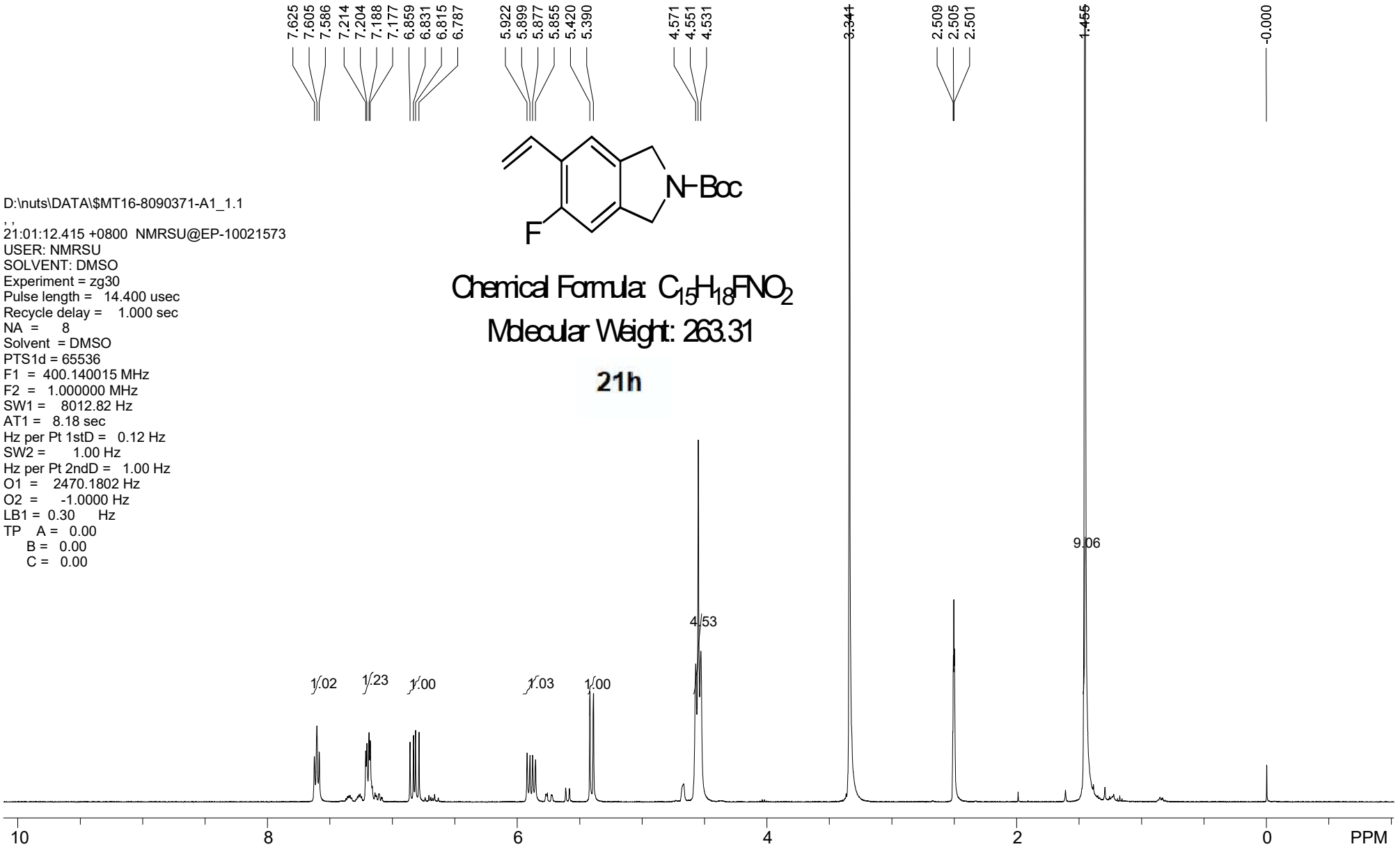

7.817
7.800
7.716
7.703
7.023
7.018
6.996
6.990
6.981
6.975
6.953
6.947
5.937
5.908
5.894
5.866
5.510
5.480
4.659
4.638
4.615
4.581
1.467
N-Boc
F3C
D:\nuts\DATA\$MT16-8090308-A1_1.1
, ,
18:32:03.809 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.139984 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2471.9800 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
Chemical Formula: C16H18F3NO2
Molecular Weight: 313
19f
0.97
1.04
1.00
0.99
1.01
4.29
9.37
10
8
6
4
2
PPM

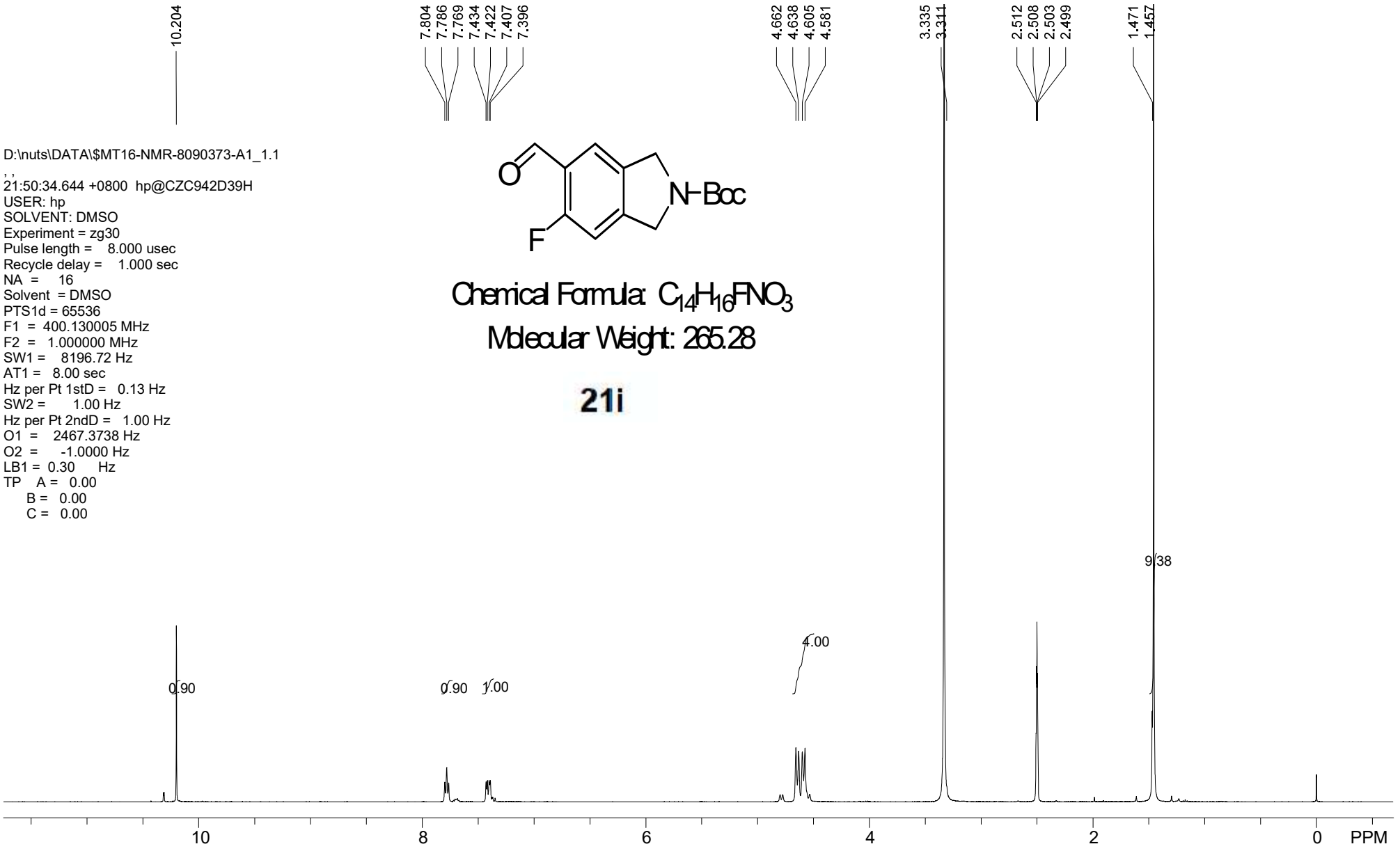

10.643
7.942
7.932
7.762
7.418
7.283
7.149
4.929
4.912
4.748
4.734
2.450
2.425
2.407
2.319
2.278
2.238
2.057
2.036
2.015
1.997
1.972
1.154
1.134
1.120
1.107
1.087
0.496
0.459
0.437
0.393
0.366
0.299
0.147
0.124
0.111
0.087
-0.000
D:\nuts\DATA\$MT16-NMR-8090318-A1_1.1
, ,
10:55:00.075 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 32
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2469.4756 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
O
HN
NH
O
O
N
CF3
CHF2
Chemical Formula: C19H18F5N3O3
Molecular Weight: 431.36
19
0.96
2.01
0.99
1.00
2.01
2.01
2.15
2.01
1.01
3.14
0.99
10
8
6
4
2
0
PPM

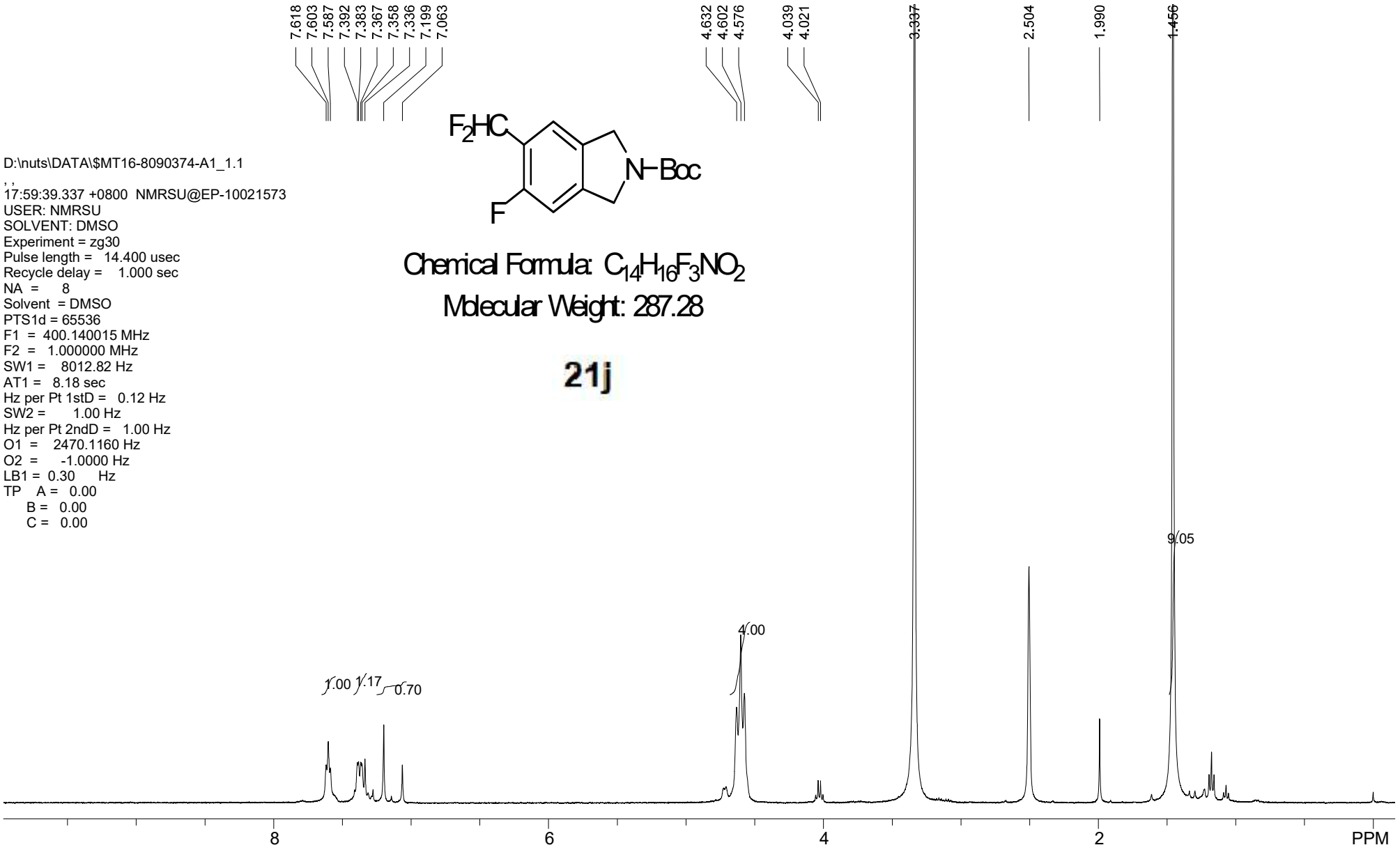

10.619
8.357
7.748
7.692
7.429
7.420
4.829
4.813
4.650
4.642
3.338
2.502
2.443
2.443
2.399
2.381
2.329
2.298
2.268
2.240
2.227
2.029
2.009
1.989
1.136
1.116
1.094
1.082
0.492
0.455
0.443
0.401
0.378
0.362
0.339
0.316
0.143
0.129
0.120
0.106
0.083
-0.000
D:\NUTS\DATA\$MT16-NMR-8090213-A1_1.1
, ,
11:17:12.656 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 32
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2468.9080 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
O
HN
NH
O
O
N
CF3
Chemical Formula: C19H20F3N3O3
Molecular Weight: 395.38
20
0.90
0.30
1.10
0.97
0.96
1.98
1.98
3.35
2.00
2.01
1.00
3.00
1.00
10
8
6
4
2
0
PPM

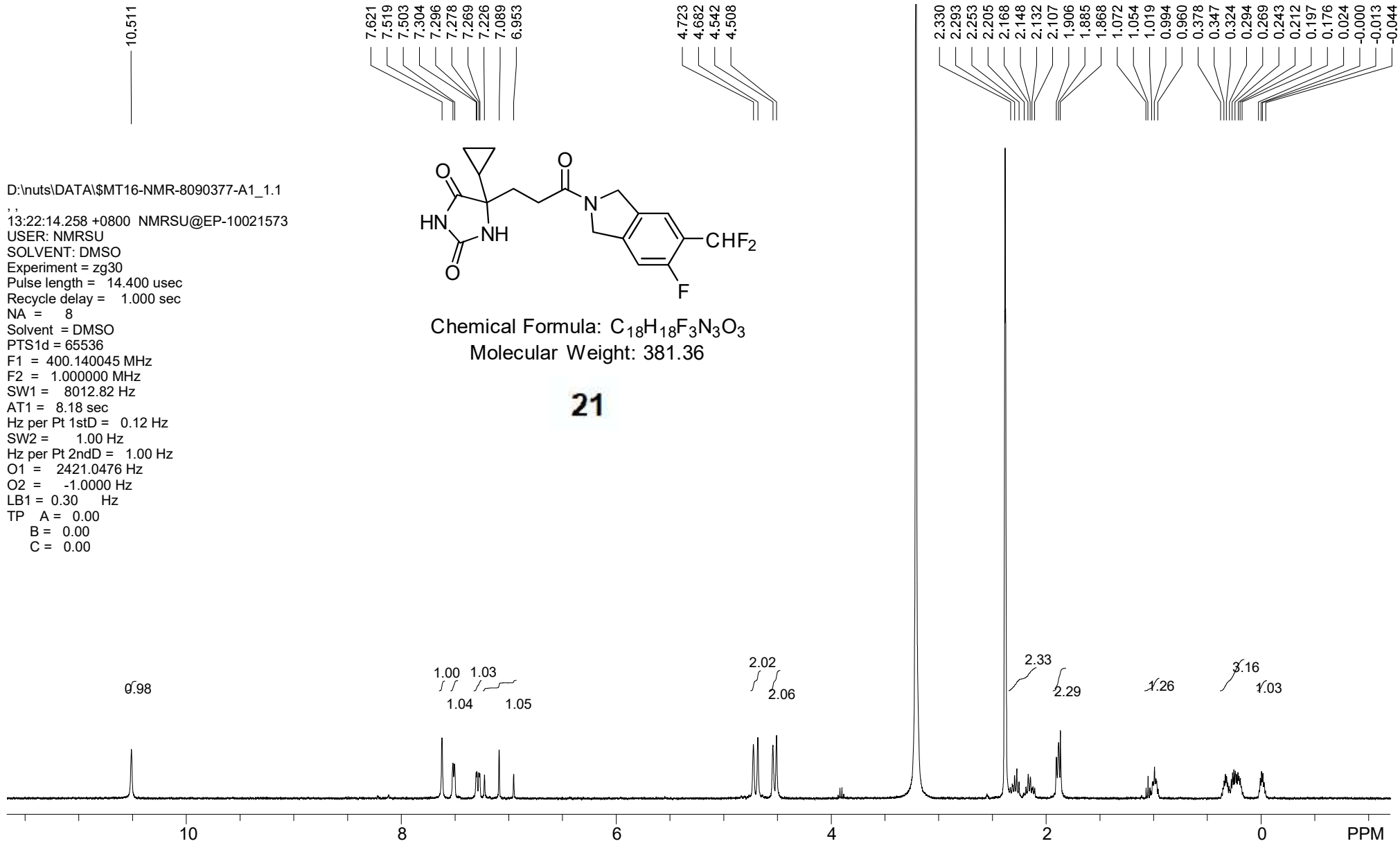

D:\nuts\DATA\$MT16-8090365-L2_1.1
, ,
09:11:03.610 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.139984 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2471.6404 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
7.631
7.615
7.339
7.317
4.278
4.245
4.190
4.129
4.106
3.171
2.512
2.508
2.504
Br
NH
F
Chemical Formula: C8H7BrFN
Molecular Weight: 216.05
21f
4.34
1.00
1.10
9
8
7
6
5
4
3
2
1
0
PPM

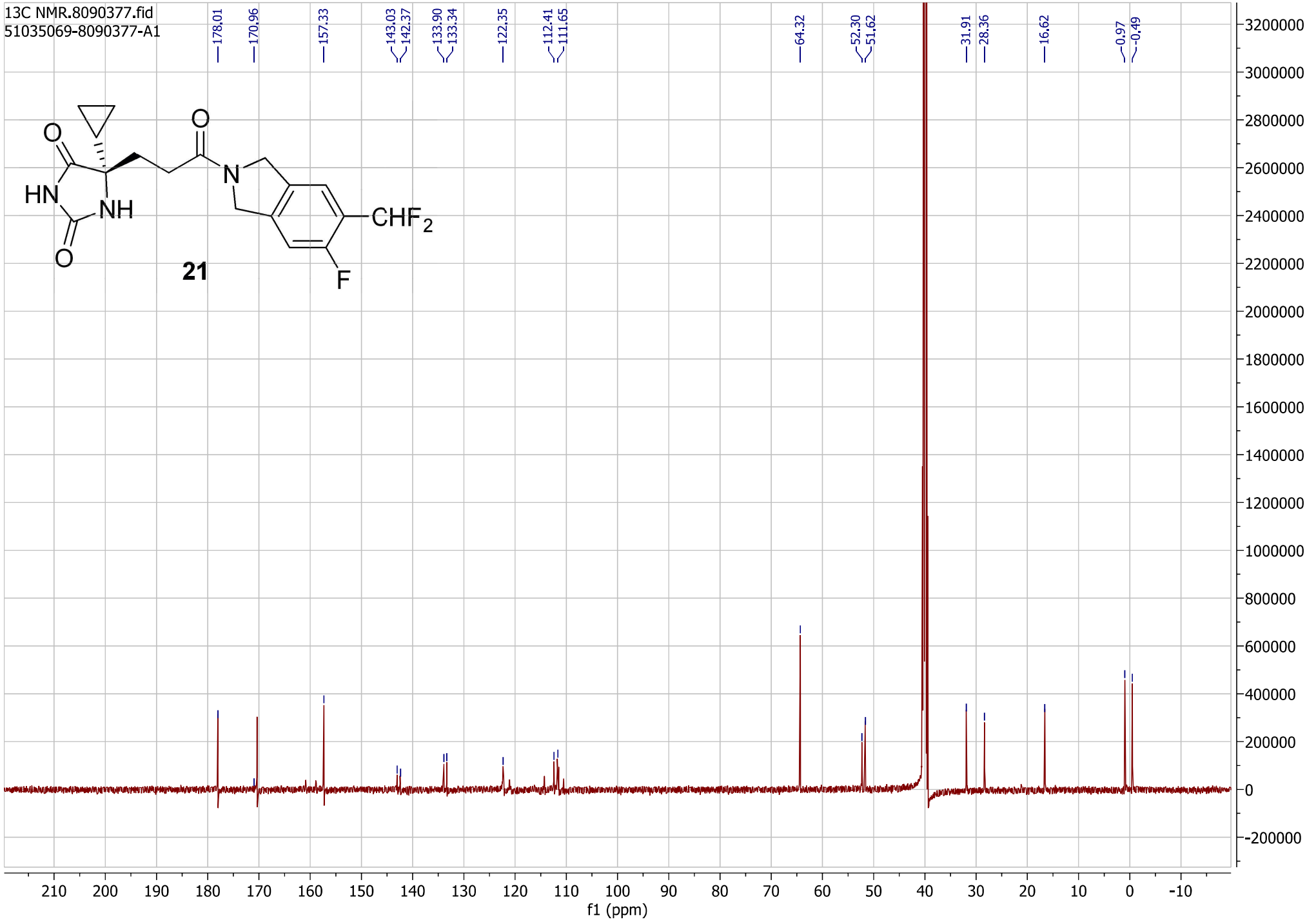

7.700
7.685
7.670
7.393
7.381
7.370
7.359
4.547
4.528
3.338
2.509
2.505
2.500
2.496
1.465
1.460
1.445
Br
N-Boc
F
D:\nuts\DATA\$MT16-8090369-A1_1.1
, ,
17:42:04.591 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2467.7410 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
Chemical Formula: C13H15BrFNO2
Molecular Weight: 316.17
21g
1.00
1.22
4.72
9.10
9
8
7
6
5
4
3
2
1
0
PPM

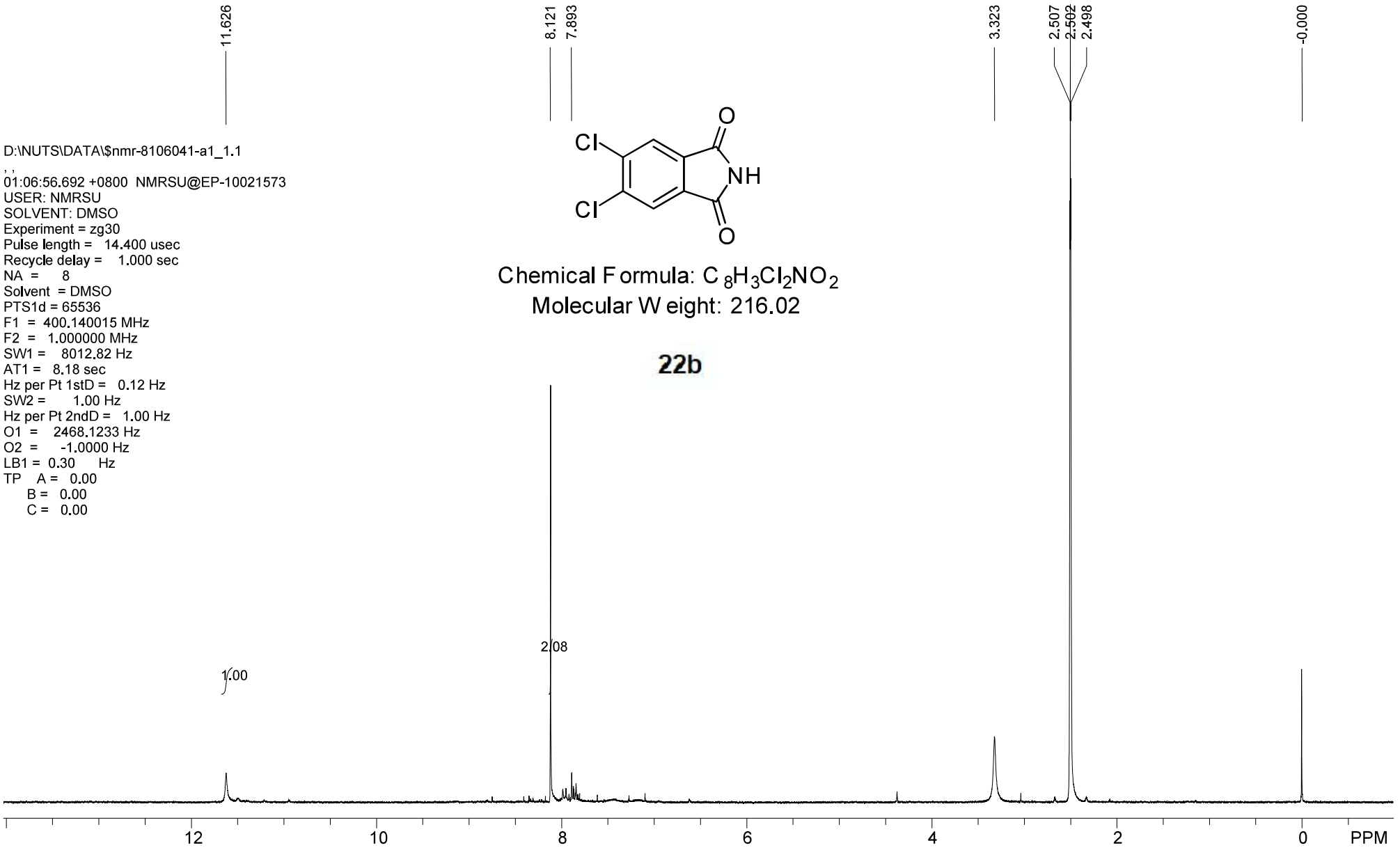

7.625
7.605
7.586
7.214
7.204
7.188
7.177
6.859
6.831
6.815
6.787
5.922
5.899
5.877
5.855
5.420
5.390
4.571
4.551
4.531
3.341
2.509
2.505
2.501
1.455
-0.000
N-Boc
F
Chemical Formula: C15H18FNO2
Molecular Weight: 263.31
21h
D:\nuts\DATA\$MT16-8090371-A1_1.1
, ,
21:01:12.415 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2470.1802 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
1.02
1.23
1.00
1.03
1.00
4.53
9.06
10
8
6
4
2
0
PPM

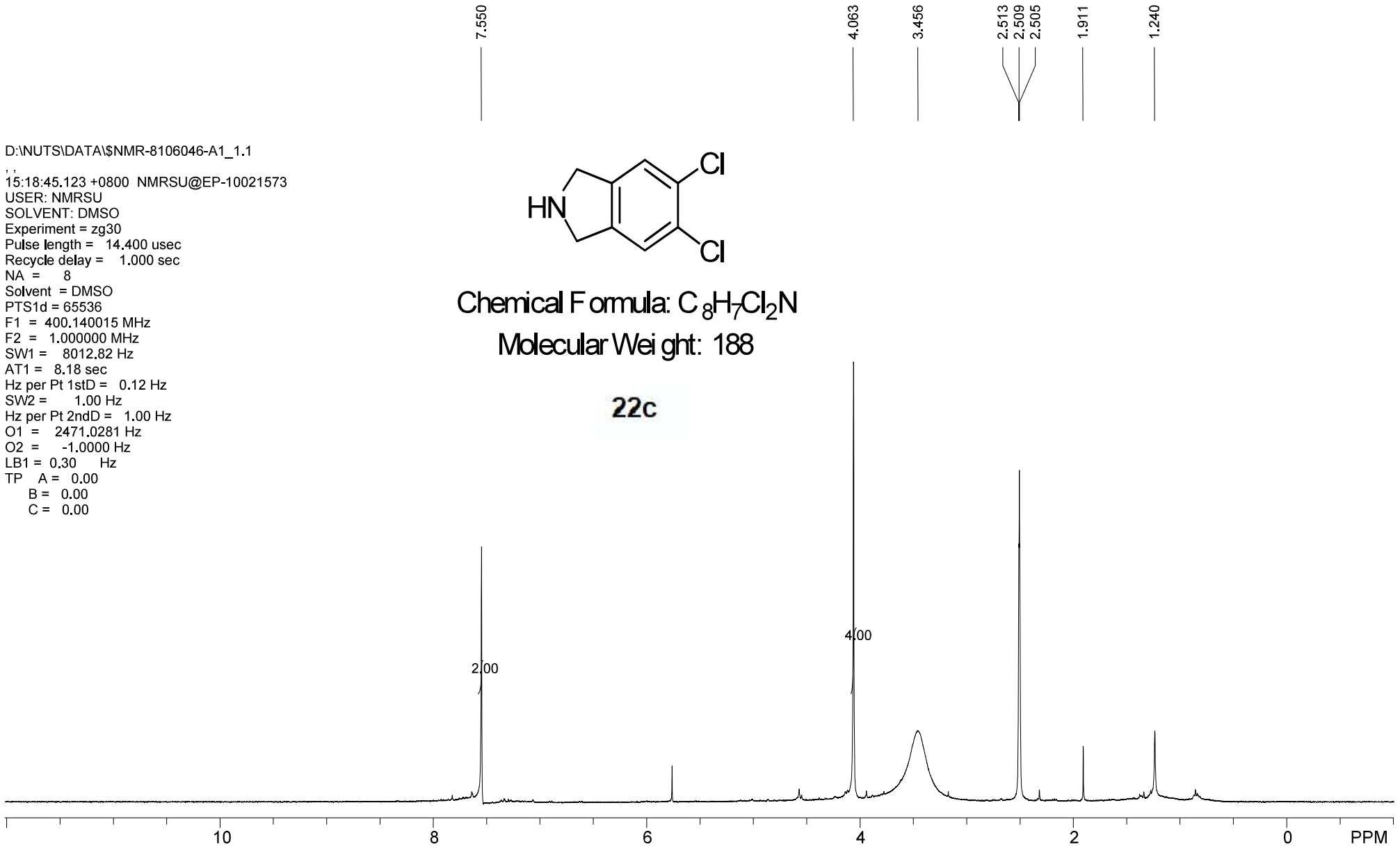

10.204
7.804
7.786
7.769
7.434
7.422
7.407
7.396
4.662
4.638
4.605
4.581
3.335
3.311
2.512
2.508
2.503
2.499
1.471
1.457
D:\nuts\DATA\$MT16-NMR-8090373-A1_1.1
, ,
21:50:34.644 +0800 hp@CZC942D39H
USER: hp
SOLVENT: DMSO
Experiment = zg30
Pulse length = 8.000 usec
Recycle delay = 1.000 sec
NA = 16
Solvent = DMSO
PTS1d = 65536
F1 = 400.130005 MHz
F2 = 1.000000 MHz
SW1 = 8196.72 Hz
AT1 = 8.00 sec
Hz per Pt 1stD = 0.13 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2467.3738 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
O
N-Boc
F
Chemical Formula: C14H16FNO3
Molecular Weight: 265.28
21i
0.90
0.90
1.00
4.00
9.38
10
8
6
4
2
0
PPM

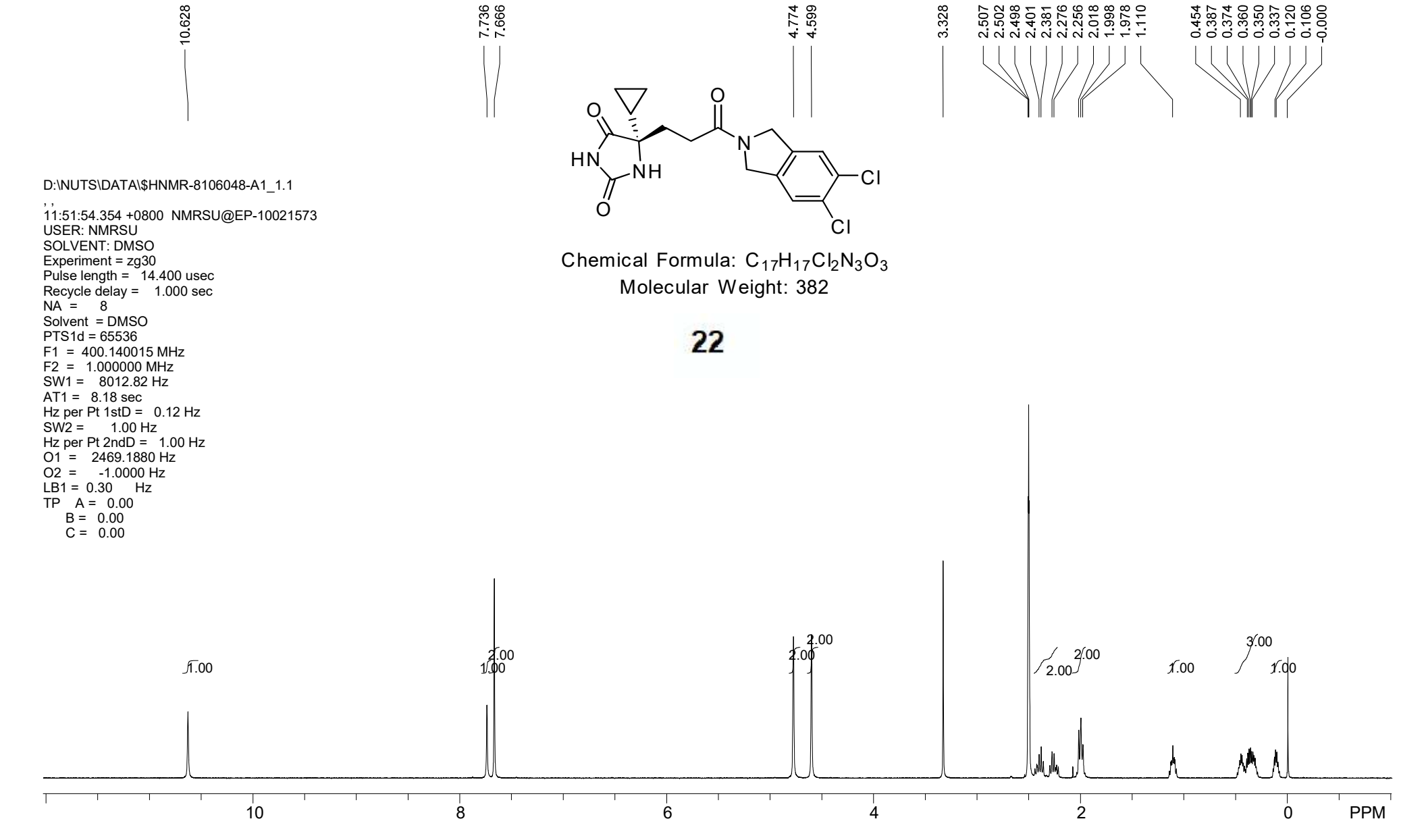

7.618
7.603
7.587
7.392
7.383
7.367
7.358
7.336
7.199
7.063
4.632
4.602
4.576
4.039
4.021
3.337
2.504
1.990
1.456
F2HC
N–Boc
F
D:\nuts\DATA\$MT16-8090374-A1_1.1
, ,
17:59:39.337 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2470.1160 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
Chemical Formula: C14H16F3NO2
Molecular Weight: 287.28
21j
1.00
1.17
0.70
4.00
9.05
8
6
4
2
PPM

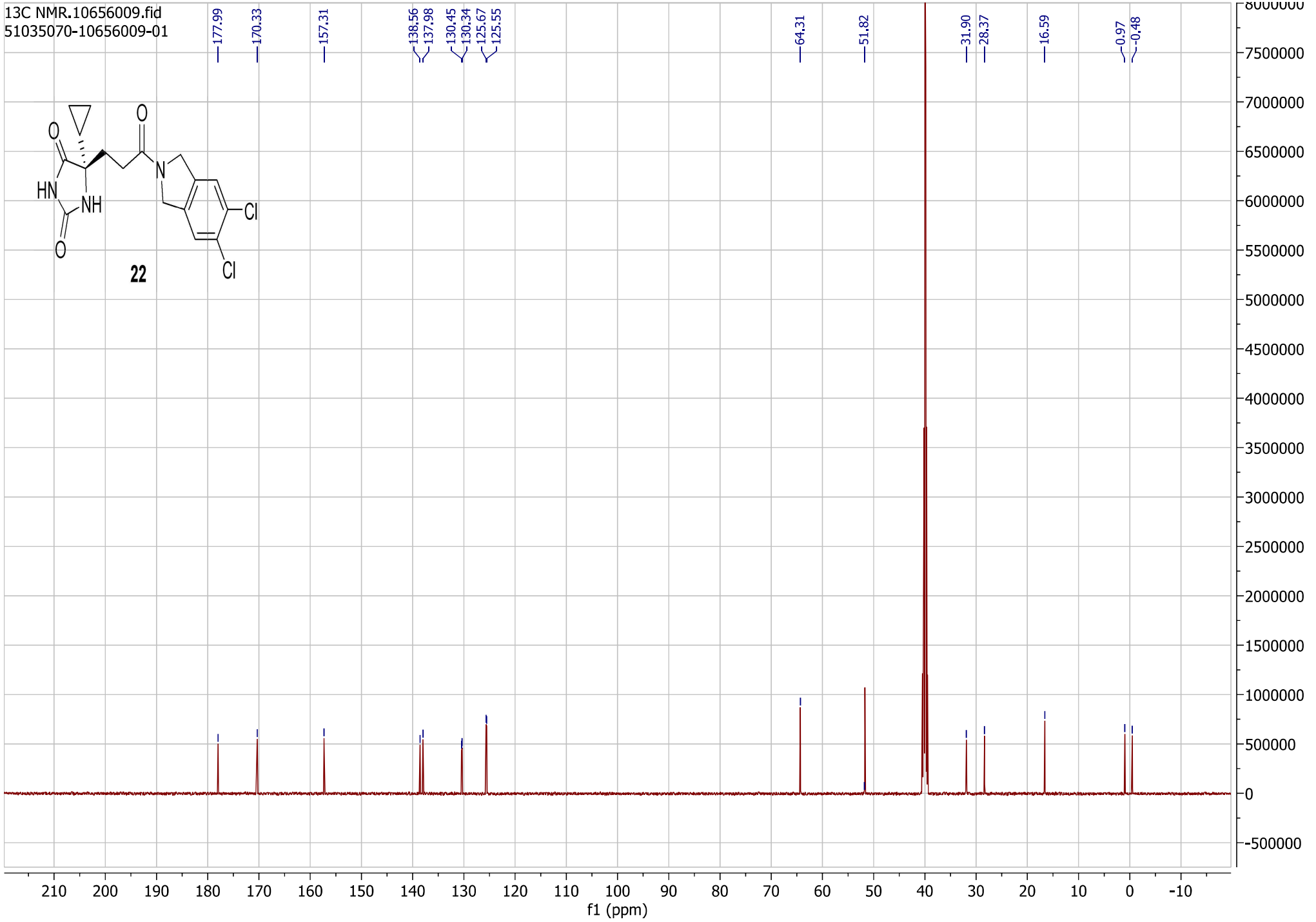

10.511
7.621
7.519
7.503
7.304
7.296
7.278
7.269
7.226
7.089
6.953
4.723
4.682
4.542
4.508
2.330
2.293
2.253
2.205
2.168
2.148
2.132
2.107
1.906
1.885
1.868
1.072
1.054
1.019
0.994
0.960
0.378
0.347
0.324
0.294
0.269
0.243
0.212
0.197
0.176
0.024
-0.000
-0.013
-0.044
D:\nuts\DATA\$MT16-NMR-8090377-A1_1.1
, ,
13:22:14.258 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.140045 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2421.0476 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
O
HN
NH
O
O
N
CHF2
F
Chemical Formula: C18H18F3N3O3
Molecular Weight: 381.36
21
0.98
1.00
1.03
1.04
1.05
2.02
2.06
2.33
2.29
1.26
3.16
1.03
10
8
6
4
2
0
PPM


13C NMR.8090377.fid
51035069-8090377-A1
178.01
170.96
157.33
143.03
142.37
133.90
133.34
122.35
112.41
111.65
64.32
52.30
51.62
31.91
28.36
16.62
0.97
-0.49
O
HN
NH
O
O
N
CHF2
F
21
3200000
3000000
2800000
2600000
2400000
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
-200000
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

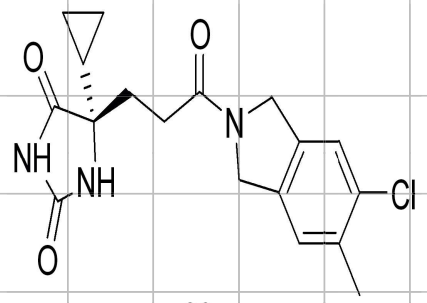

11.626
8.121
7.893
3.323
2.507
2.502
2.498
-0.000
D:\NUTS\DATA\$nmr-8106041-a1_1.1
, ,
01:06:56.692 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2468.1233 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
Cl
Cl
O
NH
O
Chemical Formula: C 8H3Cl2NO2
Molecular W eight: 216.02
22b
2.08
1.00
12
10
8
6
4
2
0
PPM


7.550
4.063
3.456
2.513
2.509
2.505
1.911
1.240
D:\NUTS\DATA\$NMR-8106046-A1_1.1
, ,
15:18:45.123 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2471.0281 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
HN
Cl
Cl
Chemical Formula: C8H7Cl2N
Molecular Weight: 188
22c
2.00
4.00
10
8
6
4
2
0
PPM


10.628
7.736
7.666
4.774
4.599
3.328
2.507
2.502
2.498
2.401
2.381
2.276
2.256
2.018
1.998
1.978
1.110
0.454
0.387
0.374
0.360
0.350
0.337
0.120
0.106
-0.000
O
HN
NH
O
O
N
Cl
Cl
D:\NUTS\DATA\$HNMR-8106048-A1_1.1
, ,
11:51:54.354 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2469.1880 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
Chemical Formula: C17H17Cl2N3O3
Molecular Weight: 382
22
1.00
2.00
1.00
2.00
2.00
2.00
2.00
1.00
3.00
1.00
10
8
6
4
2
0
PPM

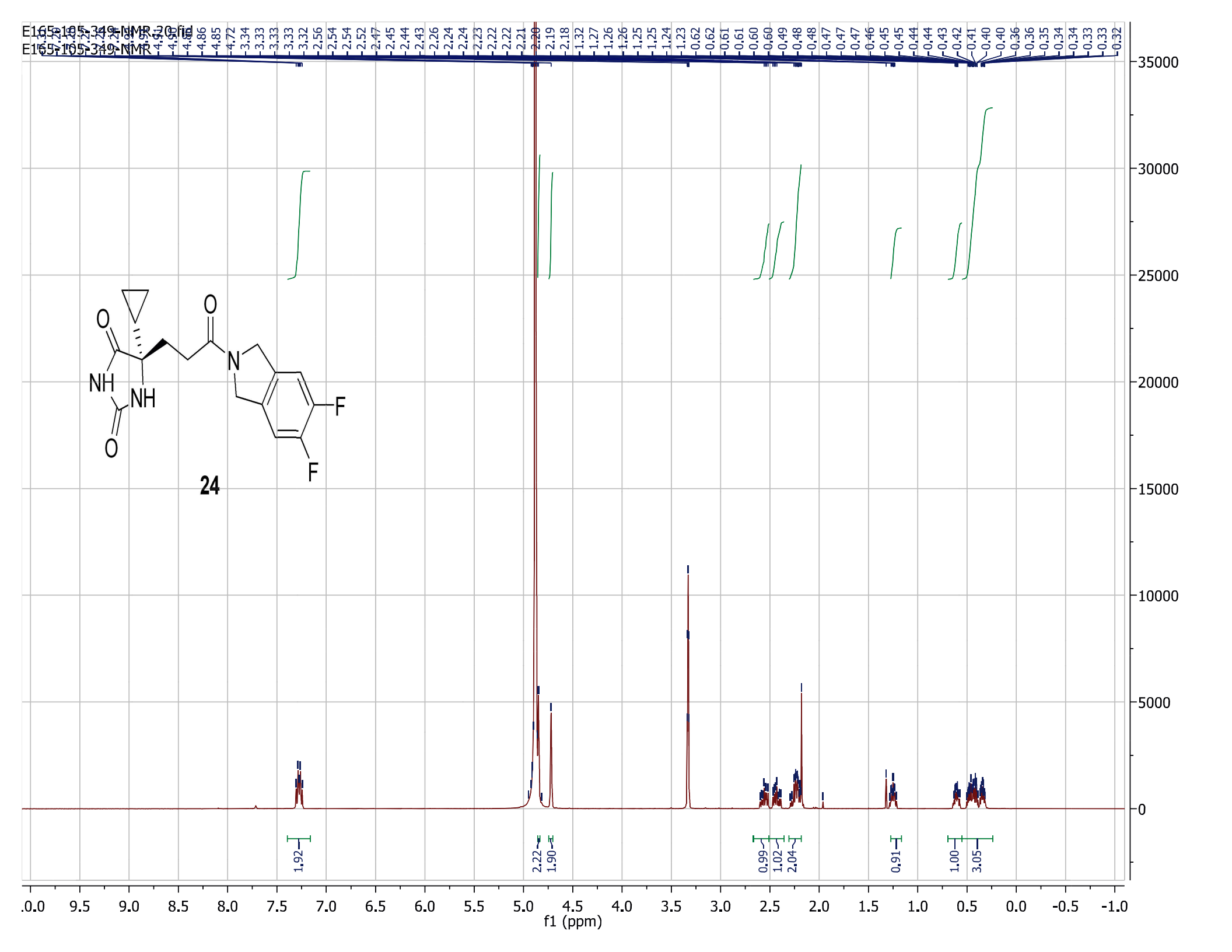

13C NMR.10656009.fid
51035070-10656009-01
177.99
170.33
157.31
138.56
137.98
130.45
130.34
125.67
125.55
64.31
51.82
31.90
28.37
16.59
0.97
-0.48
O
O
HN
NH
O
O
N
Cl
Cl
22
f1 (ppm)

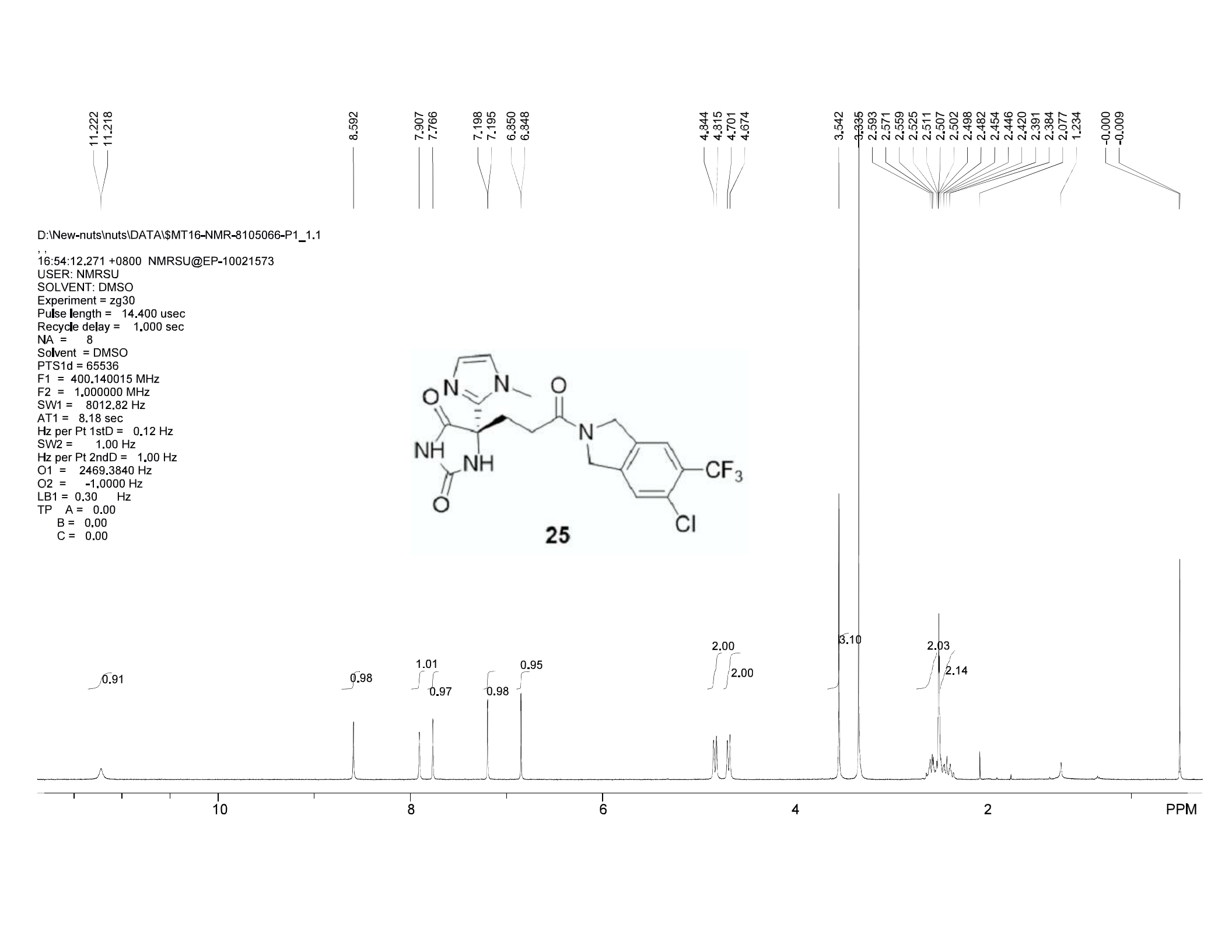
E180-24-362-NMR.10.fid
E180-24-362-NMR

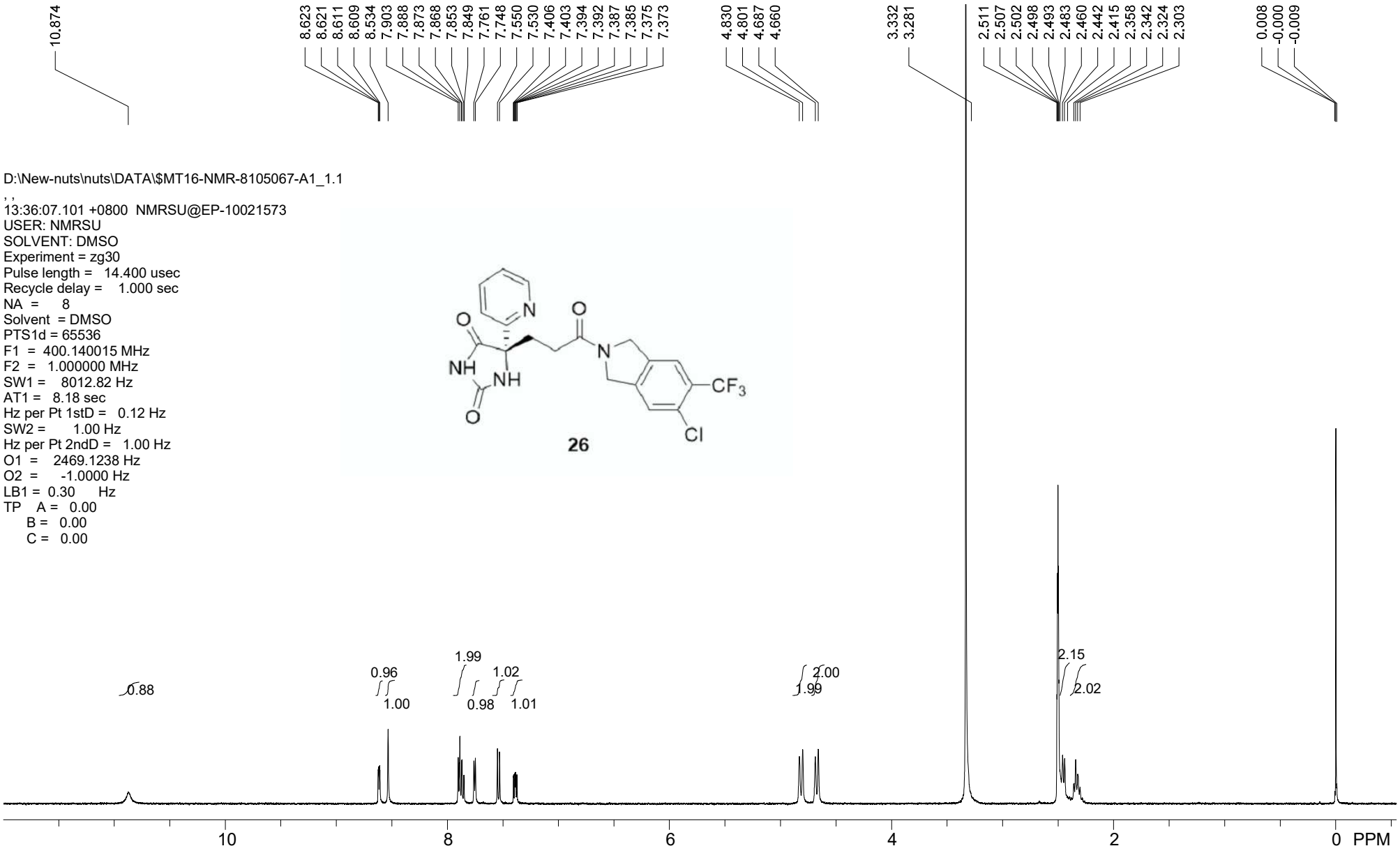


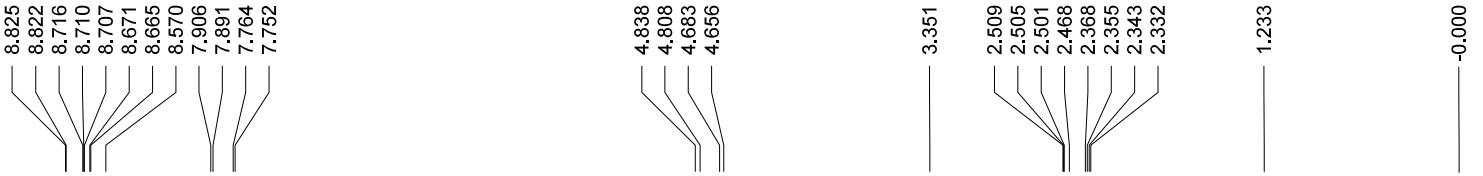
23

f1 (ppm)


E165-165-349-NMR-20.fid
E165-165-349-NMR
O
O
NH
NH
O
N
F
F
24
1.92
2.22
1.90
0.99
1.02
2.04
0.91
1.00
3.05
f1 (ppm)


11.222
11.218
8.592
7.907
7.766
7.198
7.195
6.850
6.848
4.844
4.815
4.701
4.674
3.542
3.335
2.593
2.571
2.559
2.525
2.511
2.507
2.502
2.498
2.482
2.454
2.446
2.420
2.391
2.384
2.077
1.234
-0.000
-0.009
D:\New-nuts\nuts\DATA\$MT16-NMR-8105066-P1_1.1
, ,
16:54:12.271 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2469.3840 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
N
N
O
O
NH
NH
O
N
CF3
Cl
25
0.91
0.98
1.01
0.97
0.98
0.95
2.00
2.00
3.10
2.03
2.14
10
8
6
4
2
PPM


10.874
8.623
8.621
8.611
8.609
8.534
7.903
7.888
7.873
7.868
7.853
7.849
7.761
7.748
7.550
7.530
7.406
7.403
7.394
7.392
7.387
7.385
7.375
7.373
4.830
4.801
4.687
4.660
3.332
3.281
2.511
2.507
2.502
2.498
2.493
2.483
2.460
2.442
2.415
2.358
2.342
2.324
2.303
0.008
-0.000
-0.009
D:\New-nuts\nuts\DATA\$MT16-NMR-8105067-A1_1.1
, ,
13:36:07.101 +0800 NMRSU@EP-10021573
USER: NMRSU
SOLVENT: DMSO
Experiment = zg30
Pulse length = 14.400 usec
Recycle delay = 1.000 sec
NA = 8
Solvent = DMSO
PTS1d = 65536
F1 = 400.140015 MHz
F2 = 1.000000 MHz
SW1 = 8012.82 Hz
AT1 = 8.18 sec
Hz per Pt 1stD = 0.12 Hz
SW2 = 1.00 Hz
Hz per Pt 2ndD = 1.00 Hz
O1 = 2469.1238 Hz
O2 = -1.0000 Hz
LB1 = 0.30 Hz
TP A = 0.00
B = 0.00
C = 0.00
26
0.88
0.96
1.00
1.99
0.98
1.02
1.01
2.00
1.99
2.15
2.02
10
8
6
4
2
0 PPM



D:\New-nuts\nuts\DATA\$MT16-NMR-8105072-P2_1.1

, ,

18:42:44.704 +0800 NMRSU@EP-10021573

USER: NMRSU

SOLVENT: DMSO

Experiment = zg30

Pulse length = 14.400 usec

Recycle delay = 1.000 sec

NA = 8

Solvent = DMSO

PTS1d = 65536

F1 = 400.140015 MHz

F2 = 1.000000 MHz

SW1 = 8012.82 Hz

AT1 = 8.18 sec

Hz per Pt 1stD = 0.12 Hz

SW2 = 1.00 Hz

Hz per Pt 2ndD = 1.00 Hz

O1 = 2470.5442 Hz

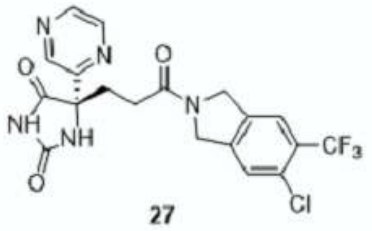
O2 = -1.0000 Hz

LB1 = 0.30 Hz

TP A = 0.00

B = 0.00

C = 0.00



27