Supporting Information

Pharmacokinetic, pharmacodynamic and metabolic characterization of a brain retentive microtubule (MT)-stabilizing triazolopyrimidine.

Anne-Sophie Cornec[a], Michael James[b], Jane Kovalevich[b], John Q. Trojanowski[b], Virginia M.-Y. Lee[b], Amos B. Smith III[a*], Carlo Ballatore[a,b*], and Kurt R. Brunden[b*]

[a] *Department of Chemistry, School of Arts and Sciences, University of Pennsylvania, 231 South 34th St., Philadelphia, PA 19104-6323*

[b] *Center for Neurodegenerative Disease Research, Institute on Aging, University of Pennsylvania, 3600 Spruce Street, Philadelphia, PA 19104-6323*

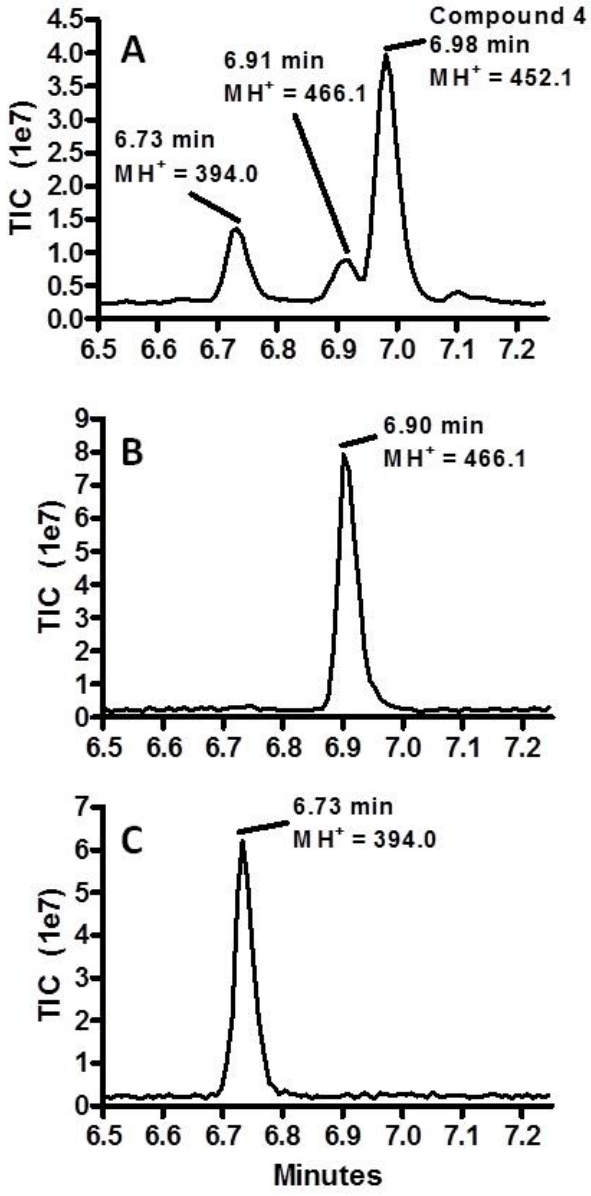


Figure S1. Comparison of LC-MS retention times and MH^+ Ions.
(A) Total Ion Chromatogram of **4** after incubation with mouse liver microsomes; (B) TIC of **5**; (C) TIC of **6**

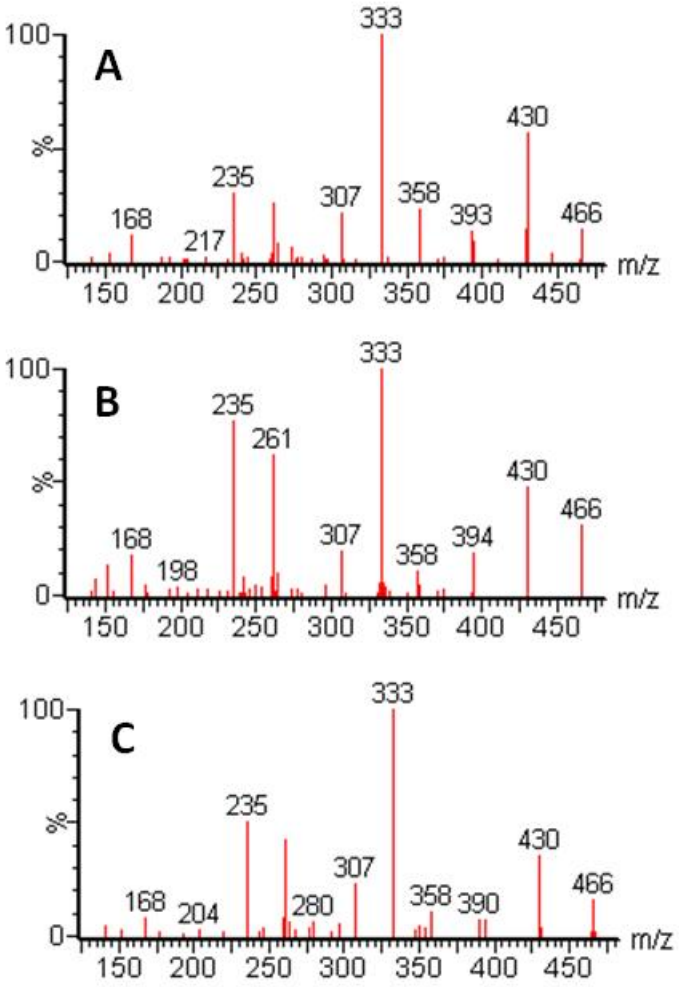


Figure S2. Comparison of collision induced fragmentation of the MH^+ = 466 parent ion. (A) Collision Induced Dissociation Spectrum of the MH^+ = 466 parent ion found after incubation of **4** with mouse liver microsomes; (B) CID Spectrum of **5**; (C) CID Spectrum of the MH^+ = 466 parent ion found in mouse plasma 30 minutes after IP administration of **4**

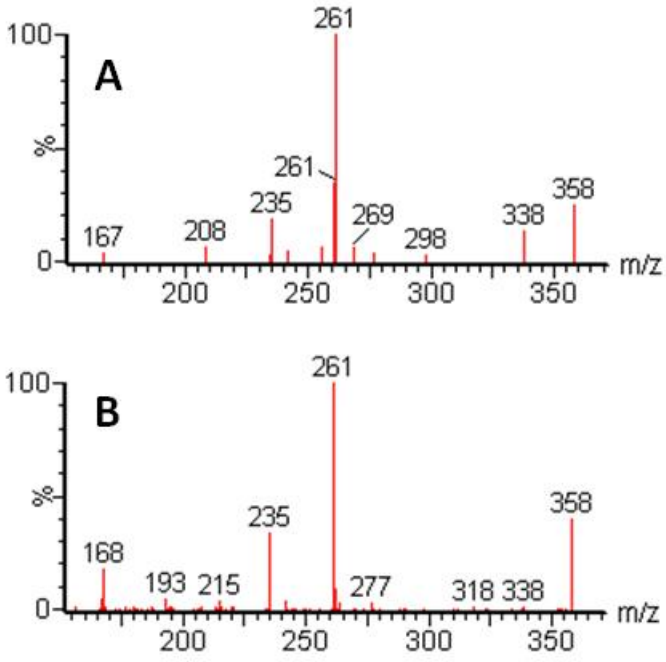
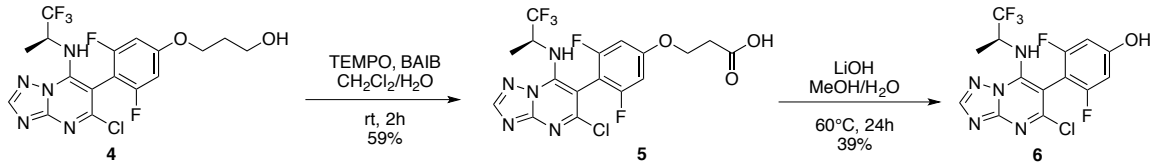


Figure S3. Comparison of collision induced fragmentation of the MH^+ = 394 parent ion (A) Collision Induced Dissociation Spectrum of the MH^+ = 394 parent ion found after incubation of **4** with mouse liver microsomes; (B) CID Spectrum of **6**

Table S1. Comparison of plasma levels of **4** 2 hours after IP or oral administration (PO) of test compound.

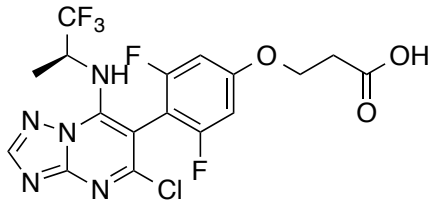
Dosing	**Plasma (nM)** **2 h Post-Dosing**
IP (5 mg/kg)	29 +/- 5
PO (10 mg/kg)	96 +/- 78

QBI293 Cellular Assays: Compound-induced changes in QBI293 cellular acetyl-tubulin levels were determined as previously described.[2, 3] The ability of compounds to elicit cellular toxicity was determined by treating QBI293 cells with varying concentrations of test compound over a 24 hour period, and assessing cell survival with Alamar Blue cell viability reagent (Invitrogen). Briefly, QBI293 cells were plated at 10,000 cells/well in a 96-well plate and allowed to grow overnight at 37˚C in 5% CO_2. The cells were subsequently treated with compounds in triplicate over a concentration range (nM-μM) that were diluted from 20 mM DMSO stocks. Tubercidin (100 μM) was used as a positive cytotoxic control, and vehicle only (0.25% DMSO) was used as a negative control. Following 18 hours of treatment, Alamar Blue reagent was added to 10% of the total well volume, and the cells were incubated for an additional 6 hours at 37˚C in 5% CO_2. Plates were then analyzed on a SprectraMax M5 fluorimeter with an excitation of 550 nm and an emission wavelength of 590 nm, with a cutoff of 570 nM. The percent cytotoxicity in compound-treated wells was calculated from the relative fluorescence units (RFUs), with the average RFUs from the tubericin positive control used to define 100% cytotoxicity and the average RFUs from the vehicle control used to define 0% toxicity.



Experimental Section

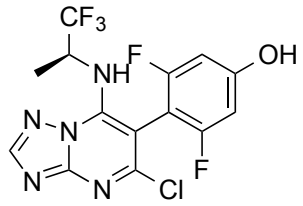
Materials and methods. All solvents were reagent grade. All reagents were purchased from Aldrich or Acros and used as received. Thin layer chromatography (TLC) was performed with 0.25 mm E. Merck pre-coated silica gel plates. TLC spots were detected by viewing under a UV light. Infrared (IR) spectra were recorded on a Jasco Model FT/IR-480 Plus spectrometer. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker AMX-500 spectrometer. Chemical shifts were reported relative to solvents. High-resolution mass spectra were measured at the University of Pennsylvania Mass Spectrometry Center on either a VG Micromass 70/70H or VG ZAB-E spectrometer. Analytical reverse-phased (Sunfire™ C18; 4.6x50 mm, 5 mL) high-performance liquid chromatography (HPLC) was performed with a Waters binary gradient module 2525 equipped with Waters 2996 PDA and Waters micromass ZQ. All samples were analyzed employing a linear gradient from 10% to 90% of acetonitrile in water over 8 minutes and flow rate of 1 mL/min, and unless otherwise stated, the purity level was >95%. Preparative reverse phase HPLC purifications were performed on a Gilson instrument (*i.e.*, Gilson 333 pumps, a 215 liquid handler, 845Z injection module, and PDA detector) employing Waters SunFire™ preparative C_{18} OBD™ columns (5 mm 19x50 or 19x100 mm). Purifications were carried out employing a linear gradient from 10% to 90% of acetonitrile in water for 15 minutes with a flow rate of 20 mL/min. Unless otherwise stated, all final compounds were found to be >95% as determined by HPLC/MS and NMR.



(*S*)-3-(4-(5-chloro-7-((1,1,1-trifluoropropan-2-yl)amino)-[1,2,4]triazolo[1,5-*a*]pyrimidin-6-yl)-3,5-difluorophenoxy)propanoic acid (**5**).

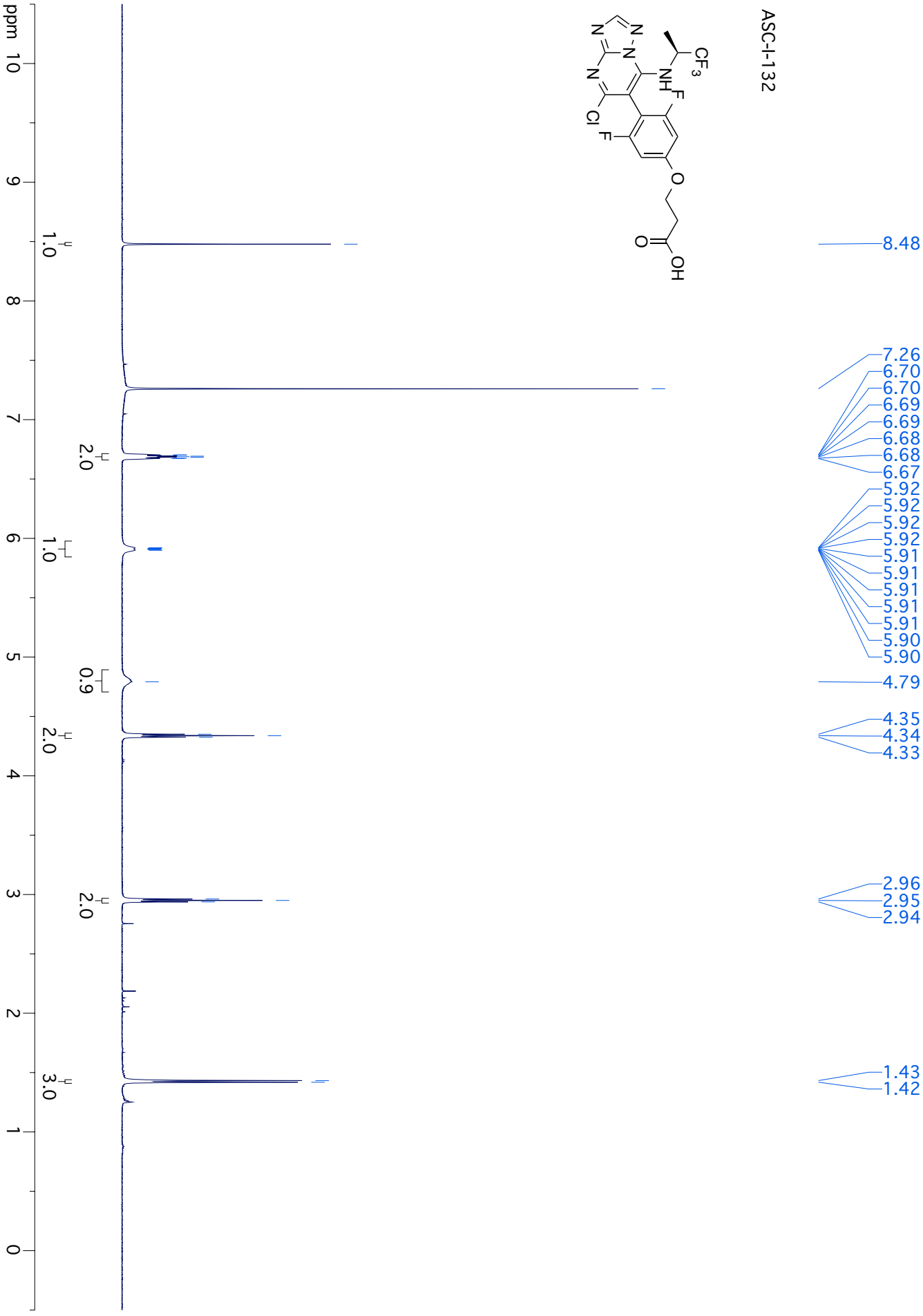
To a solution of **4** (20 mg, 0.044 mmol, prepared as described in Lou et al.[1]) in a mixture CH_2Cl_2/H_2O (90/70 μL) were added TEMPO (2 mg, 0.013 mmol) and bis(acetoxy)iodobenzene (28.5 mg, 0.088 mmol). The reaction mixture was stirred at room temperature for 2h, then the solvents were evaporated. The crude mixture was purified by preparative reverse phase HPLC to obtain the title compound as a white solid (12 mg, 59% yield).

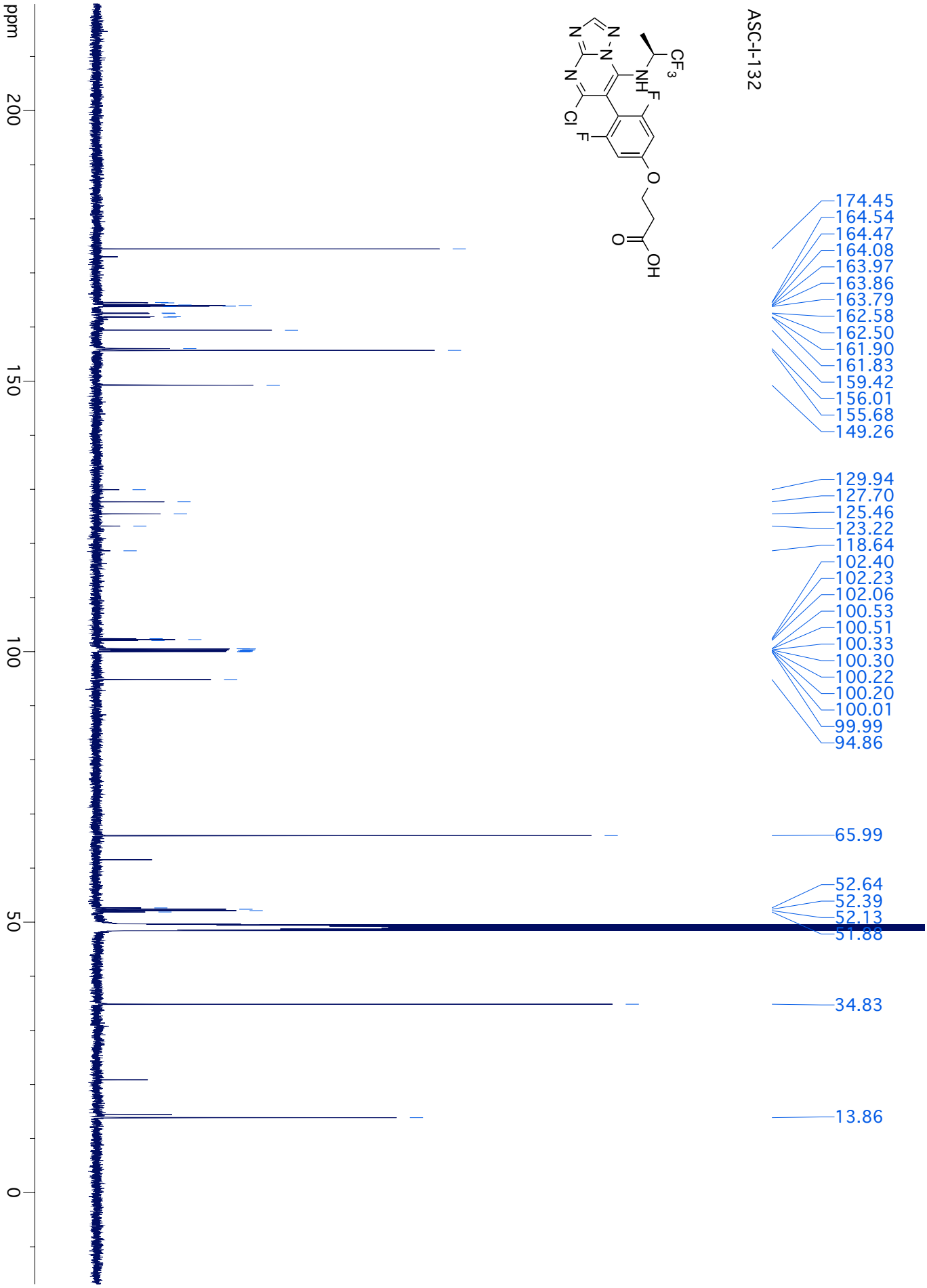
^{1}H-NMR (500 MHz; $CDCl_3$): δ 8.48 (s, 1H), 6.70-6.67 (m, 2H), 5.92-5.90 (m, 1H), 4.79 (s, 1H), 4.34 (t, *J* = 6.0 Hz, 2H), 2.95 (t, *J* = 6.0 Hz, 2H), 1.43 (d, *J* = 6.8 Hz, 3H) ppm. ^{13}C-NMR (126 MHz; MeOD): δ 174.45, 163.52 (dd, *J* = 247.2, 9.1 Hz), 163.97 (t, *J* = 14.1 Hz), 162.85 (dd, *J* = 247.3, 9.2 Hz), 159.42, 156.01, 155.68, 149.26, 126.58 (q, *J* = 281.7 Hz), 102.23 (t, *J* = 21.3 Hz), 100.42 (dd, *J* = 26.0, 2.9 Hz), 100.11 (dd, *J* = 25.9, 2.9 Hz), 94.86, 65.99, 52.26 (q, *J* = 31.8 Hz), 34.83, 13.86 ppm. IR: ν 3332, 2923, 2854, 1724, 1618, 1579 cm^{-1}. HRMS (ESI$^+$) calculated for $C_{17}H_{13}ClF_5N_5O_3$ [M+H$^+$]: 466.0705, found 466.0695.

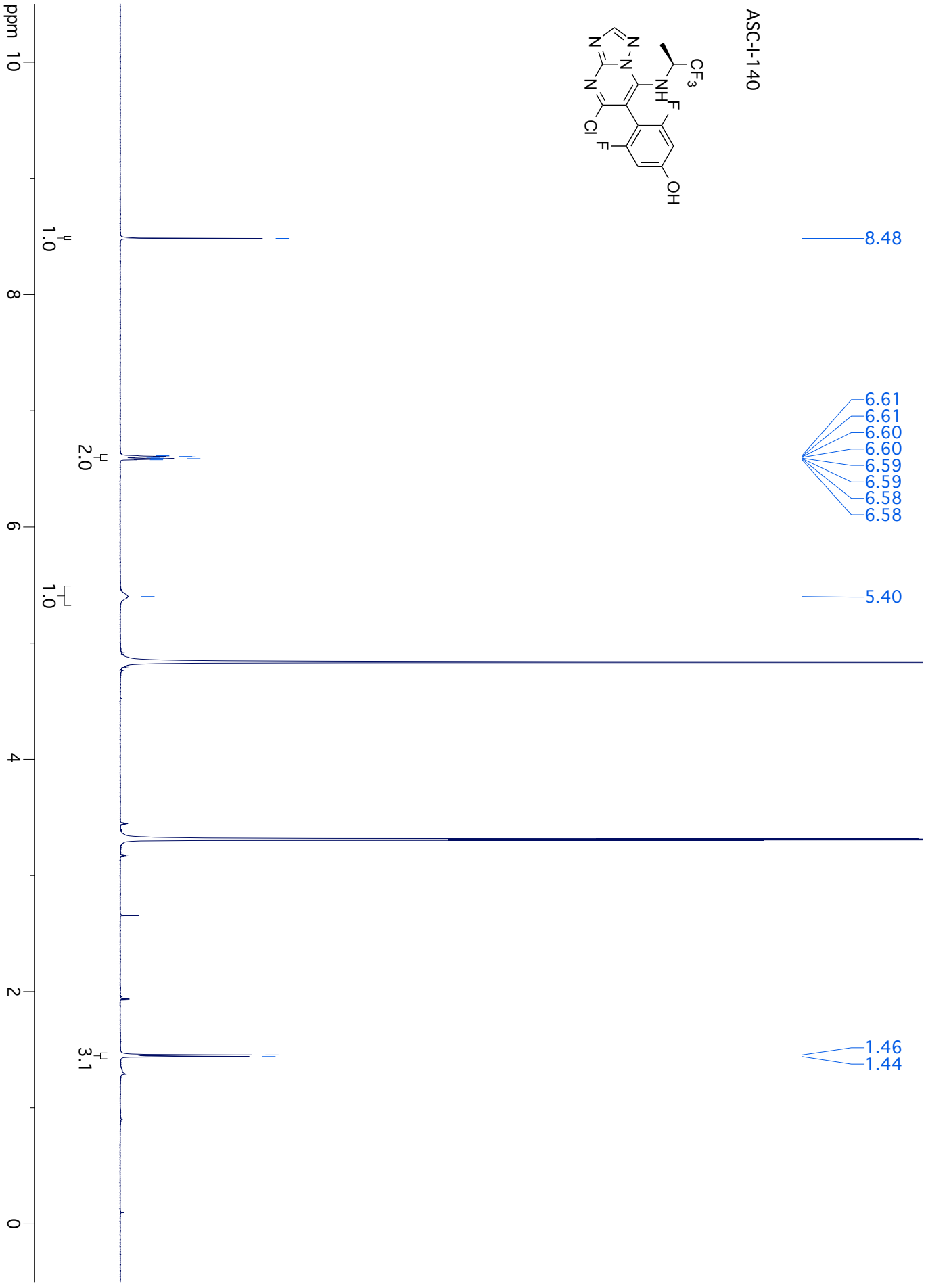


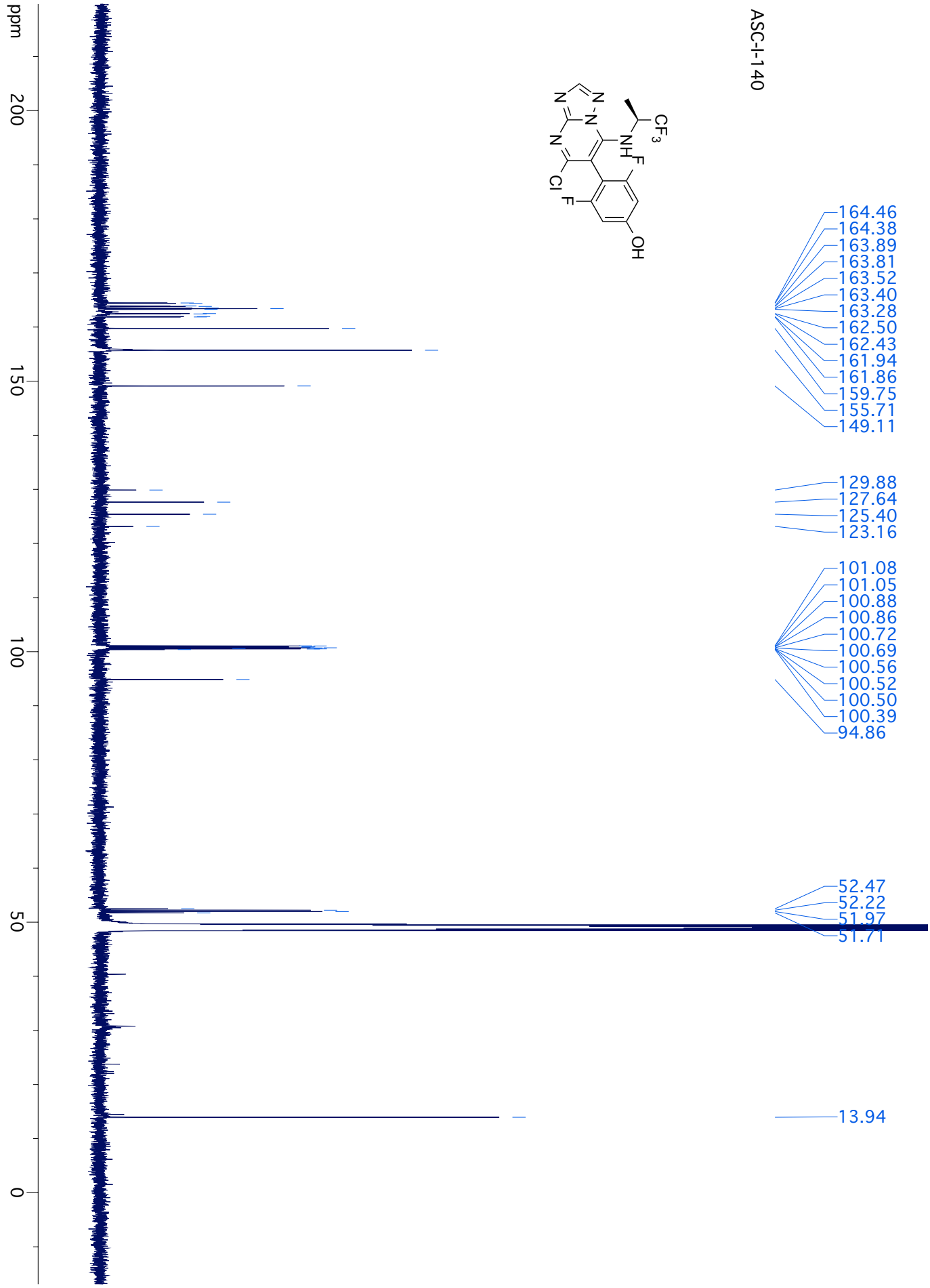
(*S*)-4-(5-chloro-7-((1,1,1-trifluoropropan-2-yl)amino)-[1,2,4]triazolo[1,5-*a*]pyrimidin-6-yl)-3,5-difluorophenol (**6**).

To a solution of **5** (11.5 mg, 0.025 mmol) in a mixture MeOH/H_2O (0.25 mL/0.25 mL), was added LiOH (11.8 mg, 0.5 mmol). The reaction mixture was stirred at 60°C for 24h, then cooled at room temperature and diluted with 5 mL of CH_2Cl_2. The organic phase was washed with HCl 1N (3 x 3 mL), and the combined aqueous layers were extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The crude products were purified by preparative reverse phase HPLC to obtain the title compound as a white solid (3.8 mg, 39% yield).
^{1}H-NMR (500 MHz; MeOD): δ 8.48 (s, 1H), 6.61-6.58 (m, 2H), 5.40 (s, 1H), 1.45 (d, *J* = 6.9 Hz, 3H) ppm. ^{13}C-NMR (126 MHz; MeOD): δ 163.44 (dd, *J* = 246.6, 9.4 Hz), 162.88 (dd, *J* = 245.8, 9.6 Hz), 163.40 (t, *J* = 14.9 Hz), 159.75, 155.71, 149.11, 126.52 (q, *J* = 281.7 Hz), 100.97 (dd, *J* = 24.7, 2.9 Hz), 100.61 (dd, *J* = 24.8, 2.9 Hz), 94.86, 52.09 (q, *J* = 31.9 Hz), 13.94 ppm. IR: ν 3345, 2920, 2848, 1622, 1583, 1458 cm^{-1}. HRMS (ESI$^-$) calculated for $C_{14}H_9ClF_5N_5O$ [M-H$^-$]: 392.0338, found 392.0340.


ASC-I-132
8.48
7.26
6.70
6.70
6.69
6.69
6.68
6.68
6.67
5.92
5.92
5.92
5.92
5.91
5.91
5.91
5.91
5.91
5.90
5.90
4.79
4.35
4.34
4.33
2.96
2.95
2.94
1.43
1.42
1.0
2.0
1.0
0.9
2.0
2.0
3.0
ppm


ASC-I-132
ppm
200
150
100
50
0
174.45
164.54
164.47
164.08
163.97
163.86
163.79
162.58
162.50
161.90
161.83
159.42
156.01
155.68
149.26
129.94
127.70
125.46
123.22
118.64
102.40
102.23
102.06
100.53
100.51
100.33
100.30
100.22
100.20
100.01
99.99
94.86
65.99
52.64
52.39
52.13
51.88
34.83
13.86
N
N
N
Cl
F
NH
CF3
F
O
O
OH


ASC-I-140
ppm
10
8
6
4
2
0
1.0
2.0
1.0
3.1
8.48
6.61
6.61
6.60
6.60
6.59
6.59
6.58
6.58
5.40
1.46
1.44
N
N
N
N
Cl
F
NH
CF3
F
OH


ASC-I-140
ppm
200
150
100
50
0
N
N
N
N
NH
CF3
F
Cl
F
OH
164.46
164.38
163.89
163.81
163.52
163.40
163.28
162.50
162.43
161.94
161.86
159.75
155.71
149.11
129.88
127.64
125.40
123.16
101.08
101.05
100.88
100.86
100.72
100.69
100.56
100.52
100.50
100.39
94.86
52.47
52.22
51.97
51.71
13.94

References

1. Lou, K.; Yao, Y.; Hoye, A. T.; James, M. J.; Cornec, A. S.; Hyde, E.; Gay, B.; Lee, V. M.-Y.; Trojanowski, J. Q.; Smith, A. B., 3rd; Brunden, K. R.; Ballatore, C. Brain-penetrant, orally bioavailable microtubule-stabilizing small molecules are potential candidate therapeutics for Alzheimer's disease and related tauopathies. *J Med Chem* **2014,** 57, 6116-6127.

2. Brunden, K. R.; Yao, Y.; Potuzak, J. S.; Ferrer, N. I.; Ballatore, C.; James, M. J.; Hogan, A. M.; Trojanowski, J. Q.; Smith, A. B. III; Lee, V. M.-Y. The characterization of microtubule-stabilizing drugs as possible therapeutic agents for Alzheimer's disease and related tauopathies. *Pharmacol. Res.* **2011,** 63, 341-351.

3. Brunden, K. R.; Gardner, N. M.; James, M. J.; Yao, Y.; Trojanowski, J. Q.; Lee, V. M. Y.; Paterson, I.; Ballatore, C.; Smith, A. B. MT-Stabilizer, Dictyostatin, Exhibits Prolonged Brain Retention and Activity: Potential Therapeutic Implications. *ACS Medicinal Chemistry Letters* **2013,** 4, 886-889.