Supplementary Information Guide

Structural basis for the action of the drug trametinib at KSR-bound MEK

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This file includes Synthetic Schemes and Procedures, including NMR and LC-MS characterization data for trametiglue, tram-bo, and biotin-linked trametinib.

Other Supplementary Information for this manuscript includes the following:

Supplementary Figure 1: Uncropped western blots

Supplementary Figure 2: Replicate data for IC_{50} determinations and washouts of MEKi via NanoBRET.

Supplementary Figure 3: Docking of trametinib into structures of isolated MEK

Supplementary Data Table 1: X-ray crystal structure data collection and refinement statistics.

Source and Replicate Data:

Source Data Fig. 4: Full *in vitro* kinome profiling data for trametiglue.

Source Data Extended Data Fig. 8: *In vitro* binding analysis via BLI, with statistical analysis, including mean and s.d. determination, for K_D, k_{on}(1/Ms), k_{dis}(1/s), and τ (min). This file also includes loading controls and extended dissociation analysis.

Source Data Extended Data Fig. 10: Source and replicate data for cell viability analysis.

Supplementary Note

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General Chemical Methods. All solvents were purchased from Sigma-Aldrich and were used as received; anhydrous solvents were used for chemical reactions, and HPLC grade solvents were used for aqueous work-ups, recrystallizations and chromatography. Reagents were purchased from various vendors and were used as received. Reactions were run as described in the individual procedures using standard double manifold and syringe techniques. Glassware was dried by baking in an oven at 130 °C for 12h prior to use, or was flame-dried. The pH of aqueous solutions was estimated using pH paper. Vacuum filtrations were carried out using a house vacuum line (~100 torr). In the individual procedures, the phrases "concentration under vacuum" and "concentrated to dryness" mean that solvent was removed on a rotary evaporator using a diaphragm pump (with an automatic vacuum regulator) and remaining traces of volatiles were removed on a high-vacuum (<1 torr) oil pump. Unless specified otherwise, the term "flask" refers to the round-bottomed variety. Reactions were monitored by TLC using EMD silica gel 60 F_{254} (250 μm) glass-backed plates (visualized by UV fluorescence quenching and stained with basic $KMnO_4$ solution) and by liquid chromatography-tandem mass spectrometry (LC-MS). Analysis by reverse-phase LC-MS was carried out on a Waters Acquity I-Class UPLC system, with a C18 column (2.1 x 30 mm; 1.7 μm particle size), heated at 50 °C, eluted at 0.6 mL/min, and using a 3 min linear gradient method with a mobile phase consisting of water/acetonitrile (0.1% v/v formic acid added to each): 95:5→1:99(0-2.5 min), then 1:99(2.5-3 min). Sample runs were monitored using alternating positive/negative electrospray ionization (50-1000 amu) and UV detection at 254 nm. Automated preparative normal- and reverse-phase chromatography was carried out with an Interchim PuriFlash 450 purification system with a diode array detector (runs were monitored at 220-400 nm). Pre-packed silica gel cartridges (12, 25 and 40 g; 15 μm particle size) were employed for normal-phase (silica gel) chromatography, eluting at 20-30 mL/min. Preparative reverse-phase chromatography was carried out with an Agilent 1260 Infinity using a C18 column (30 x 100 mm; 5 μm particle size) with a multiwavelength detector, eluting at 40 mL/min with a pressure limit of 200 bar; crude samples were injected with an autosampler, typically in a 90:10 mixture of MeOH/DMSO (1.5 mL/injection). Carbon-decoupled ^{1}H NMR spectra were recorded at 400 MHz on a Bruker spectrometer and are reported in ppm using the residual solvent signal (dimethylsulfoxide-d_6 = 2.50 ppm; methanol-d_4 = 3.31 ppm) as an internal standard. Data are reported as: {(shift), [(s=singlet, d=doublet, dd=doublet of doublets, ddd=doublet of a doublet of doublets, t=triplet, dt=doublet of triplets, q=quartet, m=multiplet, br=broad, ap=apparent), (*J*=coupling constant in Hz), (integration)]}. Proton-decoupled ^{13}C NMR spectra were recorded at 100 MHz on a Bruker spectrometer and are reported in ppm using the residual solvent signal (dimethylsulfoxide-d_6 = 39.5 ppm) as an internal standard. ^{19}F NMR spectra were recorded at 376 MHz on a Bruker spectrometer and are reported in ppm using added $CFCl_3$ (0.00 ppm) as an internal standard; compounds with only one signal were integrated relative to a known amount of the internal standard.

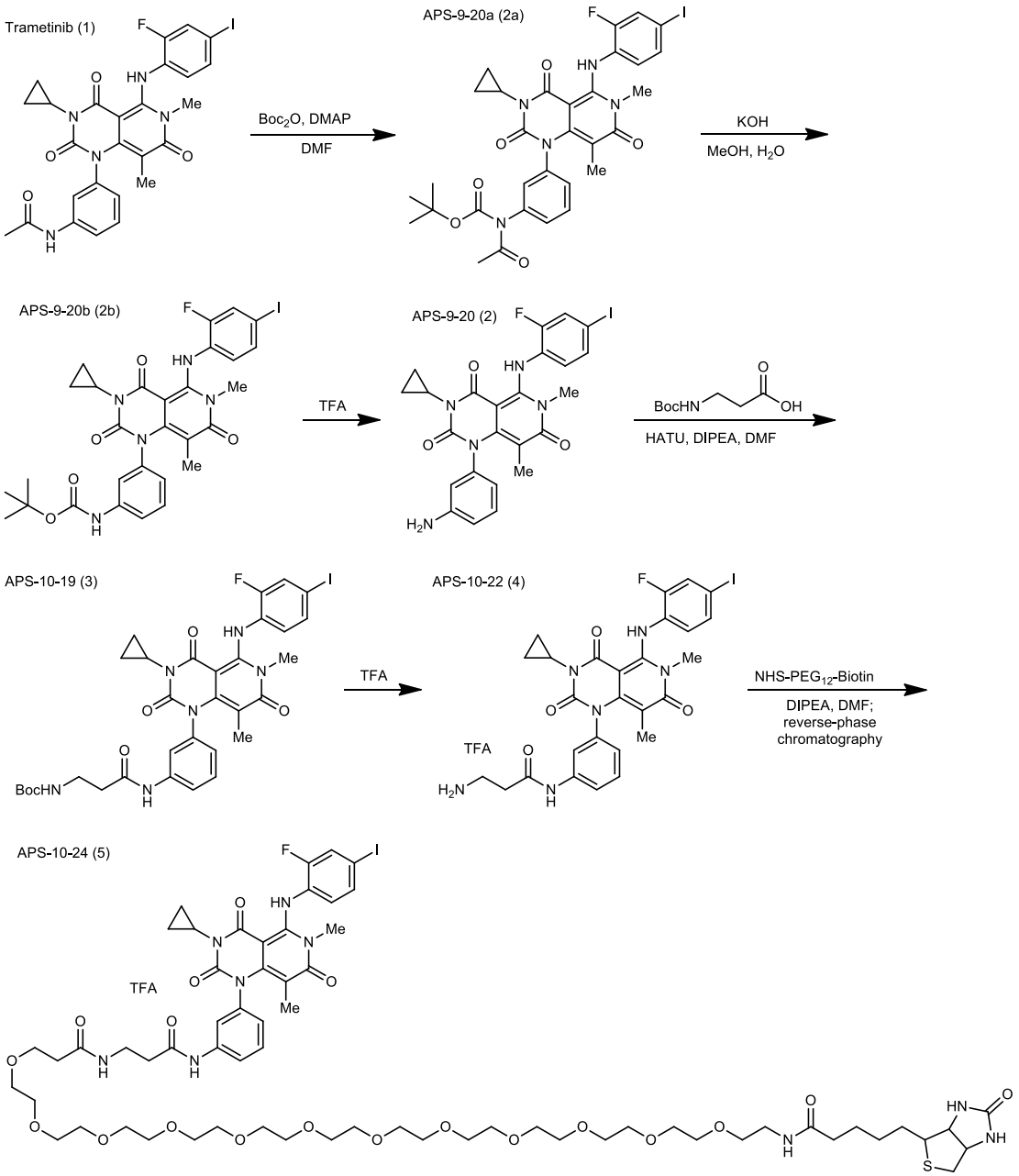


Figure 1. Synthetic route to trametinib-PEG_{12}-biotin (**5**) from trametinib (**1**).

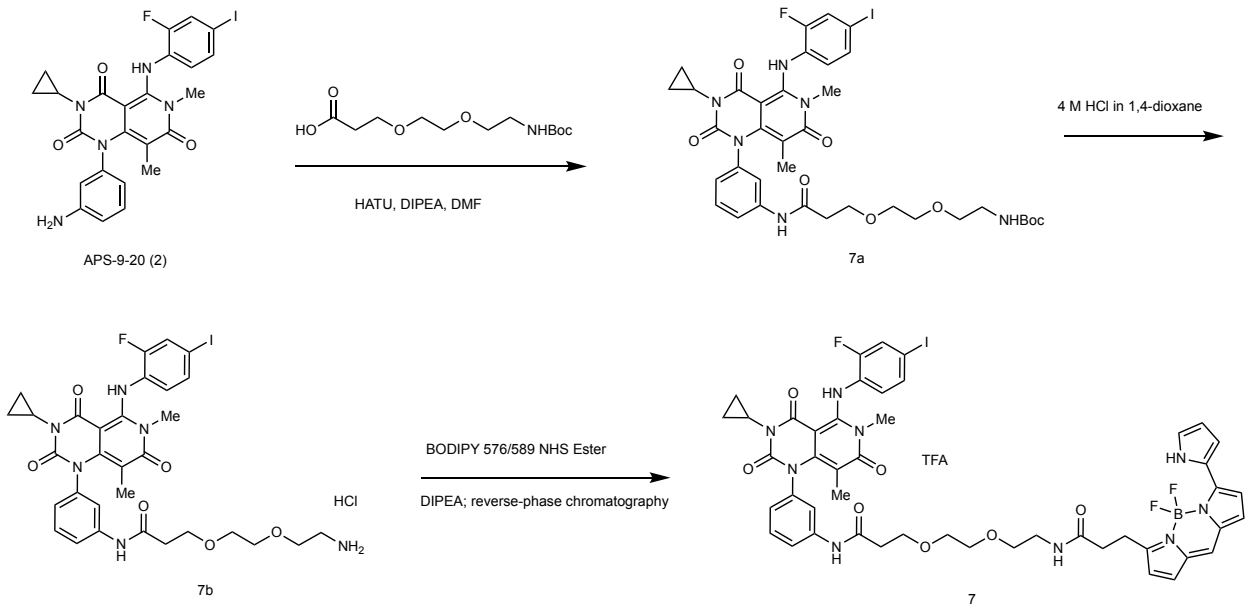
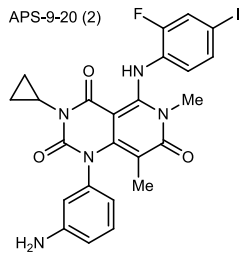
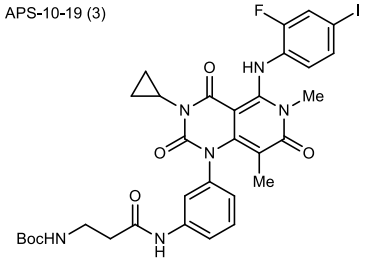


Figure 2. Synthetic route to Tram-bo (**7**) from APS-9-20 (**2**)



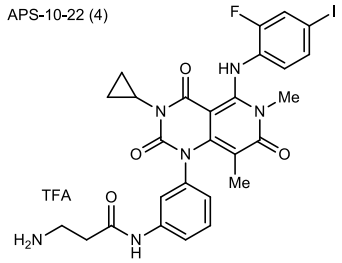
1-(3-Aminophenyl)-3-cyclopropyl-5-((2-fluoro-4-iodophenyl)amino)-6,8dimethylpyrido[4,3-*d*]pyrimidine-2,4,7(1*H*,3*H*,6*H*)-trione (2; APS-9-20).

To a solution of trametinib (**1**; 250 mg, 0.406 mmol), 4-(dimethylamino)pyridine (DMAP; 99.2 mg, 0.812 mmol) and DMF (dimethylformamide; 3 mL), in an 8 mL vial, was added a solution of di-*tert*-butyl dicarbonate (Boc_2O; 266 mg, 1.22 mmol) and DMF (1.5 mL) dropwise over 1 min. The vial was sealed under Ar and the solution was stirred for 1 h. The solution was transferred to a 50 mL flask and concentrated to dryness. The remaining solid (**2a**) was dissolved in MeOH (3 mL) and then an aqueous solution of KOH (2.0 mL, 1.0 M solution, 2.0 mmol) was added. The solution was stirred for 4 h, then diluted with brine (30 mL) and extracted with CH_2Cl_2 (3 x 30 mL). The organic extracts were pooled, dried (Na_2SO_4), filtered and concentrated to dryness. The remaining solid (**2b**) was dissolved in trifluoroacetic acid (TFA; 3.0 mL, 39 mmol). The solution was stirred for 30 min and then concentrated to dryness from toluene (3 x 3 mL). The remaining material was partitioned between CH_2Cl_2 (50 mL) and saturated $NaHCO_3$ solution (50 mL), and transferred to a separatory funnel. The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (25 mL). The organic extracts were pooled, dried (Na_2SO_4), filtered and concentrated to dryness. The remaining solid was purified by silica gel chromatography (25 g cartridge), eluting at 25 mL/min and using a linear gradient of CH_2Cl_2/MeOH: 100:0→90:10 over 37 column volumes to yield 189 mg (81% over 3 steps) of the title compound as an off-white solid: **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.06 (s, 1 H), 7.78 (dd, *J*=10.3, 2.0 Hz, 1 H), 7.54 (dd, *J*=8.6, 1.0 Hz, 1 H), 7.05 (t, *J*=7.8 Hz, 1 H), 6.90 (t, *J*=8.7 Hz, 1 H), 6.50 - 6.59 (m, 2 H), 6.46 (dd, *J*=7.8, 1.0 Hz, 1 H), 5.25 (s, 2 H), 3.07 (s, 3 H), 2.61 (tt, *J*=7.2, 3.7 Hz, 1 H), 1.35 (s, 3 H), 0.89 - 1.00 (m, 2 H), 0.59 - 0.69 (m, 2 H); **^{19}F NMR** (376 MHz, DMSO-d_6) δ -124.0 - -123.9 (m, 1 F); **LC-MS** (ESI+) *m/z*: $[M+H]^+$ Calcd for $C_{24}H_{22}FIN_5O_3$ 574.1; Found 574.3.



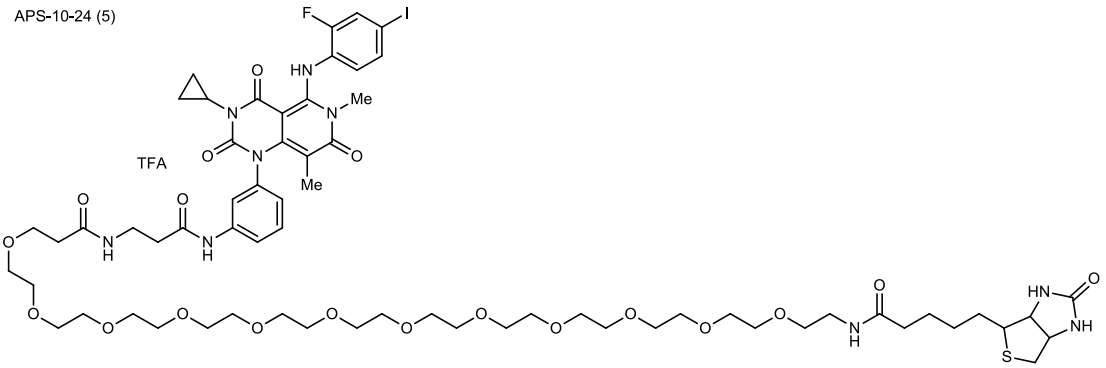
***tert*-Butyl (3-((3-(3-cyclopropyl-5-((2-fluoro-4-iodophenyl)amino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-*d*]pyrimidin-1(2*H*)-yl)phenyl)amino)-3-oxopropyl)carbamate (3; APS-10-19).**

To a solution of 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU; 58.6 mg, 0.154 mmol), *N*,*N*-diisopropylethylamine (DIPEA; 55.0 µL, 0.316 mmol) and DMF (0.5 mL), in a 4 mL vial, was added a solution of 3-((*tert*-butoxycarbonyl)amino)propanoic acid (29.1 mg, 0.154 mmol) and DMF (0.5 mL) dropwise over 1 min. The dark orange solution was stirred for 30 min and then **2** (80.0 mg, 0.140 mmol) was added in one portion. The reaction was initially heterogeneous, but became clear over 1 h, and was stirred for a total of 14 h. The solution was concentrated to dryness and purified by silica gel chromatography (25 g cartridge), eluting at 25 mL/min and using a linear gradient of CH_2Cl_2/EtOAc: 100:0→0:100 over 30 column volumes to yield 103 mg (>99%) of the title compound as an off-white solid: **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.07 (s, 1 H), 10.09 (s, 1 H), 7.79 (d, *J*=10.3 Hz, 1 H), 7.50 - 7.70 (m, 3 H), 7.36 (t, *J*=8.1 Hz, 1 H), 7.03 (d, *J*=7.8 Hz, 1 H), 6.81 - 6.98 (m, 2 H), 3.21 (q, *J*=6.4 Hz, 2 H), 3.07 (s, 3 H), 2.62 (tt, *J*=6.9, 3.8 Hz, 1 H), 2.43 - 2.51 (m, 2 H; signal overlaps with DMSO-d_5 peak), 1.37 (s, 9 H), 1.26 (s, 3 H), 0.95 (d, *J*=6.1 Hz, 2 H), 0.67 (br s, 2 H); **LC-MS** (ESI+) *m/z*: $[M+H]^+$ Calcd for $C_{32}H_{35}FIN_6O_6$ 745.2; Found 745.3.



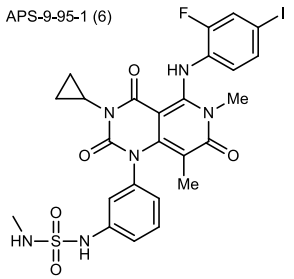
3-Amino-*N*-(3-(3-cyclopropyl-5-((2-fluoro-4-iodophenyl)amino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-*d*]pyrimidin-1(2*H*)-yl)phenyl)propanamide 2,2,2-trifluoroacetate (4; APS-10-22).

A 25 mL flask was charged with **3** (88.2 mg, 0.118 mmol) and TFA (1 mL). The solution was stirred for 1 h and then concentrated to dryness from toluene (3x3 mL). The remaining residue was purified by reverse-phase chromatography, eluting at 40 mL/min and using a linear gradient of H_2O (with 0.1% TFA)/MeCN (with 0.1% TFA): 90:10→1:99 over 20 minutes to yield 80.6 mg (>99%) of the title compound as a tan solid: **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.07 (s, 1 H), 10.32 (s, 1 H), 7.69 - 7.84 (m, 4 H), 7.66 (t, *J*=2.0 Hz, 1 H), 7.58 (dd, *J*=16.6, 8.3 Hz, 2 H), 7.39 (t, *J*=8.1 Hz, 1 H), 7.06 (dd, *J*=7.9, 1.1 Hz, 1 H), 6.92 (t, *J*=8.6 Hz, 1 H), 3.04 - 3.14 (m, 5 H), 2.71 (t, *J*=6.7 Hz, 2 H), 2.62 (tt, *J*=7.2, 3.7 Hz, 1 H), 1.25 (s, 3 H), 0.90 - 1.00 (m, 2 H), 0.61 - 0.70 (m, 2 H); **^{19}F NMR** (376 MHz, DMSO-d_6) δ -73.2 (s, 3 F), -123.9 - -123.8 (m, 1 F); **LC-MS** (ESI+) *m/z*: $[M+H]^+$ Calcd for $C_{27}H_{27}FIN_6O_4$ 645.1; Found 645.3.



***N*-(3-((3-(3-cyclopropyl-5-((2-fluoro-4-iodophenyl)amino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-*d*]pyrimidin-1(2*H*)-yl)phenyl)amino)-3-oxopropyl)-1-(5-(2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxanonatriacontan-39-amide 2,2,2-trifluoroacetate (5; APS-10-24).**

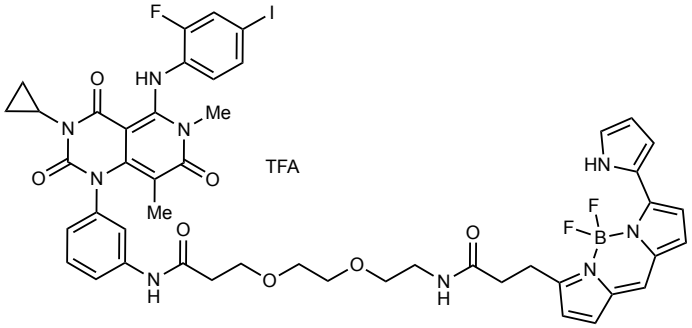
To a solution of **4** (10.0 mg, 0.015 mmol), *N*-hydroxysuccinimide-polyethylene glycol$_{12}$-biotin (NHS-PEG$_{12}$-biotin; 13.8 mg, 0.015 mmol) and DMF (0.5 mL), in a 4 mL vial, was added DIPEA (8.0 μL, 0.046 mmol). The solution was stirred for 1 h, diluted with MeOH (1.5 mL) and purified by reverse-phase chromatography, eluting at 40 mL/min and using a linear gradient of H_2O (with 0.1% TFA)/MeCN (with 0.1% TFA): 90:10→1:99 over 12 minutes to yield 17.6 mg (76%) of the title compound as a glass: **^{1}H NMR** (400 MHz, methanol-d$_4$) δ 7.73 (t, *J*=1.8 Hz, 1 H), 7.66 (dd, *J*=10.0, 1.7 Hz, 1 H), 7.53 - 7.60 (m, 2 H), 7.41 (t, *J*=8.1 Hz, 1 H), 7.11 (dd, *J*=7.9, 1.1 Hz, 1 H), 6.87 (t, *J*=8.6 Hz, 1 H), 4.49 (dd, *J*=7.8, 4.4 Hz, 1 H), 4.30 (dd, *J*=7.8, 4.4 Hz, 1 H), 3.70 (t, *J*=6.0 Hz, 2 H), 3.56 - 3.66 (m, 33 H), 3.49 - 3.56 (m, 3 H), 3.33 - 3.39 (m, 2 H), 3.20 (s, 3 H), 2.92 (dd, *J*=12.7, 5.1 Hz, 1 H), 2.67 - 2.75 (m, 2 H), 2.61 (t, *J*=6.4 Hz, 2 H), 2.44 (t, *J*=6.0 Hz, 2 H), 2.22 (t, *J*=7.3 Hz, 2 H), 1.52 - 1.80 (m, 3 H), 1.45 (q, *J*=7.6 Hz, 2 H), 1.38 (s, 2 H), 1.01 - 1.10 (m, 2 H), 0.71 - 0.80 (m, 2 H); **^{19}F NMR** (376 MHz, methanol-d$_4$) δ -75.3 (s, 3 F), -123.7 - -123.6 (m, 1 F); **LC-MS** (ESI+) *m/z*: $[M+Na]^+$ Calcd for $C_{64}H_{93}FIN_9NaO_{19}S$ 1492.5; Found 1492.7.



***N*-(3-(3-Cyclopropyl-5-((2-fluoro-4-iodophenyl)amino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-*d*]pyrimidin-1(2*H*)-yl)phenyl)methylaminosulfonamide (6; APS-9-95-1;Trametiglue).**

To a mixture of **2** (25.0 mg, 0.044 mmol) and CH_2Cl_2 (0.6 mL), in a 4 mL vial, was added methylsulfamoyl chloride (4.5 μL, 0.052 mmol), followed by pyridine (11.0 μL, 0.136 mmol). The reaction was stirred for 6 h and then was purified directly by silica gel chromatography (25 g

cartridge), eluting at 25 mL/min and using a linear gradient of : 100:0→90:10 CH_2Cl_2/MeOH over column 30 volumes to yield 16.4 mg (56%) of the title compound as an off-white solid: **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.09 (s, 1 H), 9.83 (s, 1 H), 7.79 (d, *J*=10.3 Hz, 1 H), 7.55 (d, *J*=8.1 Hz, 1 H), 7.29 - 7.46 (m, 2 H), 7.19 (d, *J*=8.3 Hz, 1 H), 7.11 (s, 1 H), 7.02 (d, *J*=7.6 Hz, 1 H), 6.92 (t, *J*=8.6 Hz, 1 H), 3.07 (s, 3 H), 2.58 - 2.67 (m, 1 H), 2.43 (d, *J*=4.9 Hz, 3 H), 1.24 (s, 3 H), 0.95 (d, *J*=5.9 Hz, 2 H), 0.66 (br s, 2 H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ 164.2, 162.8, 155.4, 152.9, 150.9, 144.7, 140.6, 139.3, 134.0, 129.0, 128.2, 128.1, 125.0, 124.7, 123.4, 118.8, 117.0, 102.0, 90.2, 88.3, 88.2, 34.0, 28.1, 24.8, 12.8, 8.2; **LC-MS** (ESI+) *m/z*: $[M+H]^+$ Calcd for $C_{25}H_{25}FIN_6O_5S$ 667.1; Found 667.2.

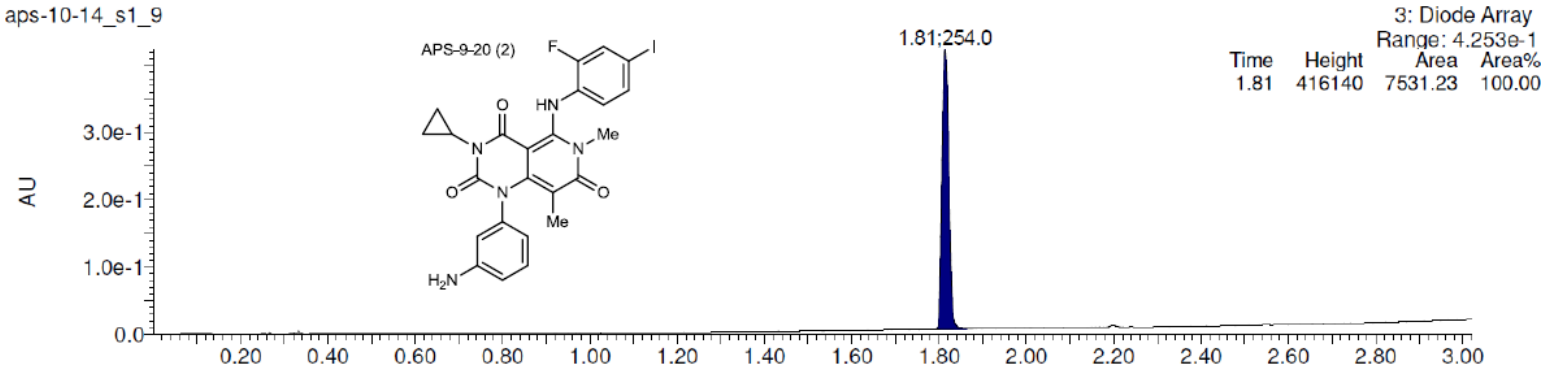


7

***N*-(3-(3-cyclopropyl-5-((2-fluoro-4-iodophenyl)amino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-*d*]pyrimidin-1(2*H*)-yl)phenyl)-3-(2-(2-(3-(5,5-difluoro-7-(1*H*-pyrrol-2-yl)-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-3-yl)propanamido)ethoxy)ethoxy)propenamide 2,2,2, trifluoroacetate (7; Tram-bo)**

To a solution of HATU (13 mg; 0.034 mmol) and DIPEA (18 μL; 0.102 mmol) in DMF (1.5 mL) was added 2,2-dimethyl-4-oxo-3,8,11-trioxa-5-azatetradecan-14-oic acid (10 mg; 0.036 mmol). The mixture was stirred on ice for 30 min before **2** (20 mg; 0.035 mmol) was added in one portion. The reaction was stirred at RT overnight and monitored by LC/MS until complettion, evaporated of DMF, and chromatographed on silica using a linear gradient of methanol/dichloromethane: 0:100→10:90 over 40 column volumes. Relevant fractions were pooled, concentrated by rotovap, and dried under high vacuum overnight. The amine (**7a**) was deprotected by direct addition of 4 M HCl in 1,4-dioxane (5 mL) under vigorous stirring for 30 min at RT. The solvent was removed, and then co-evaporated with toluene (3x10 mL) to dryness (30 mg crude-**7b**). A portion of the free amine salt (9 mg; 0.011 mmol) was directly added to a solution of DIPEA (61 μL; 0.035 mmol) in DMF (1 mL). The BODIPY 576/589 NHS ester (5 mg; 0.011 mmol; ThermoFisher D2225) dissolved in DMF was added to the amine salt, and stirred for 30 min at RT in darkness. A mixture of MeOH:DMSO (9:1; 1.5 mL) was added to the reaction, which was then purified using reverse-phase chromatography. The title compound eluted at 40 mL/min and using a linear gradient of H_2O (with 0.1% TFA)/MeCN (with 0.1% TFA): 90:10→1:99 over 25 minutes to yield 8 mg (70

%- final step) of a dark purple solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.23 (s, 1 H), 10.37 (s, 1 H), 8.66 (s, 1 H), 7.64 (s, 1 H), 7.52 (dd, *J*=9.7, 1.42 Hz, 1 H), 7.45 (d, *J*=8.2 Hz, 1 H), 7.29-7.35 (m, 2 H), 7.16-7.18 (m, 1 H), 7.06 (d, *J*=4.6 Hz, 1 H), 6.96 – 7.0 (m, 3 H), 6.88 (d, *J*=4.0 Hz, 1 H), 6.81 (d, *J*=4.0 Hz, 1 H), 6.66-6.69 (m, 2 H), 6.35-6.38 (m, 1 H), 6.27 (d, *J*=3.8 Hz, 1 H), 3.72-3.74 (m, 2 H), 3.60 (s, 4 H), 3.49-3.51 (m, 2 H), 3.37 (s, 2 H), 3.27 (t, *J*=7.4 Hz, 2 H), 3.18 (s, 3 H), 2.64-2.68 (m, 3 H), 2.57 – 2.60 (m, 2 H), 1.38 (s, 3 H), 1.26 (s, 2 H), 1.05 - 1.10 (m, 2 H), 0.76 - 0.77 (m, 2 H); **^{19}F NMR** (376 MHz, $CDCl_3$) δ -76.38 (s, 3 F-TFA), -122.44 (m,1 F), -140.15- -140.53 (m, 2 F); **LC-MS** (ESI+) *m/z*: $[M+H]^+$ Calcd for $C_{47}H_{47}BF_3IN_9O_7$ 1044.6; Found 1044.4.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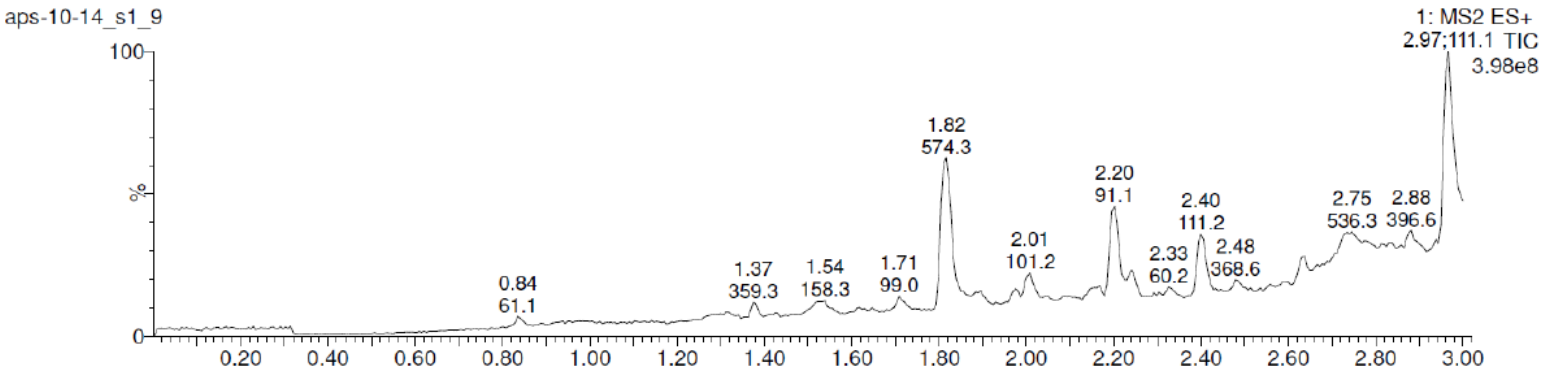
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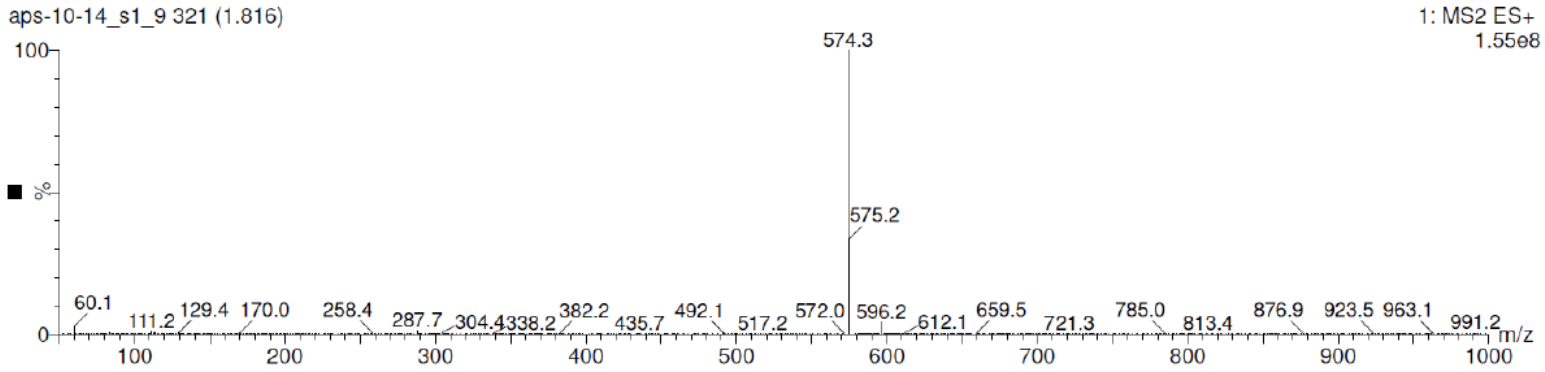
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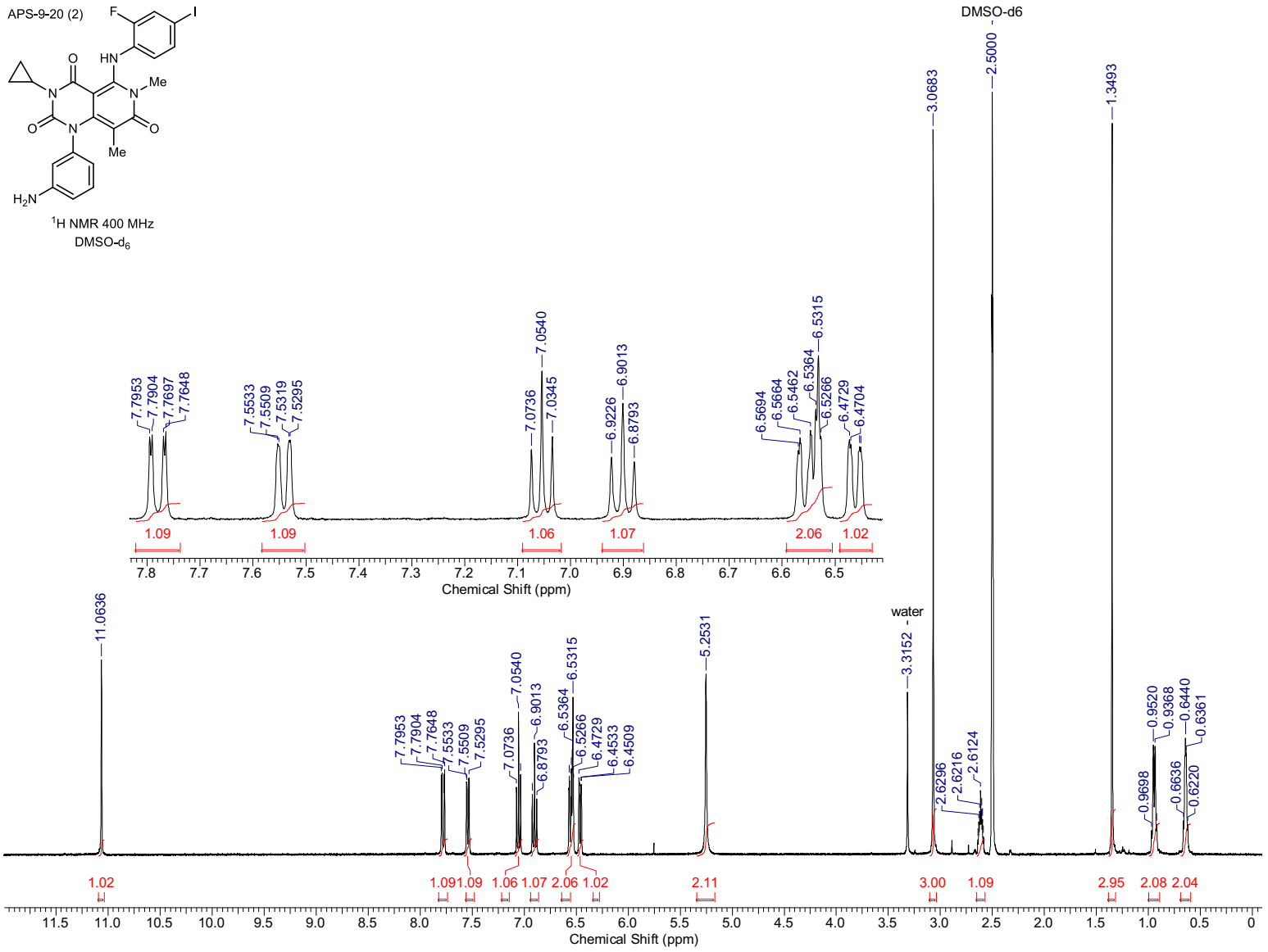
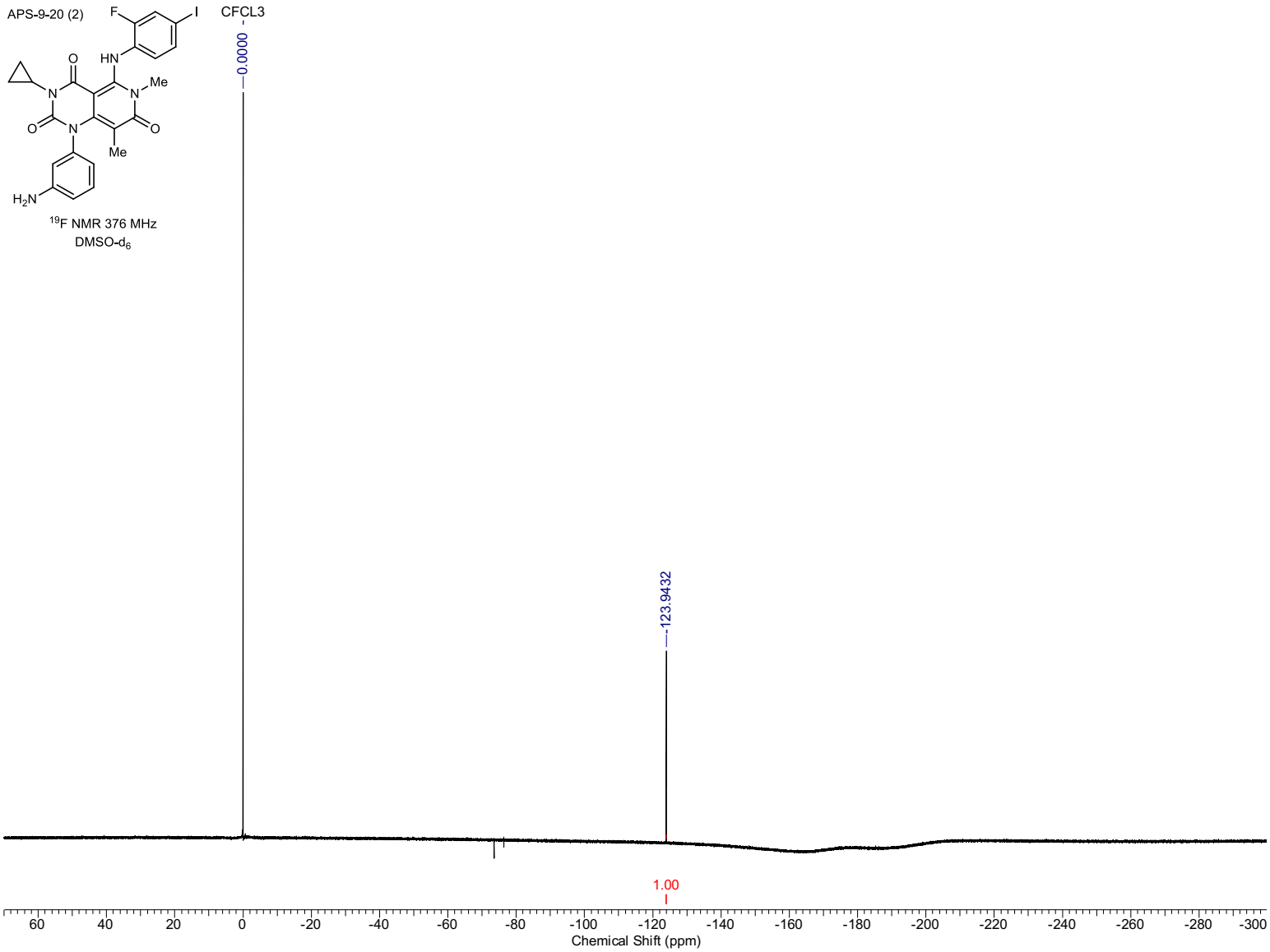
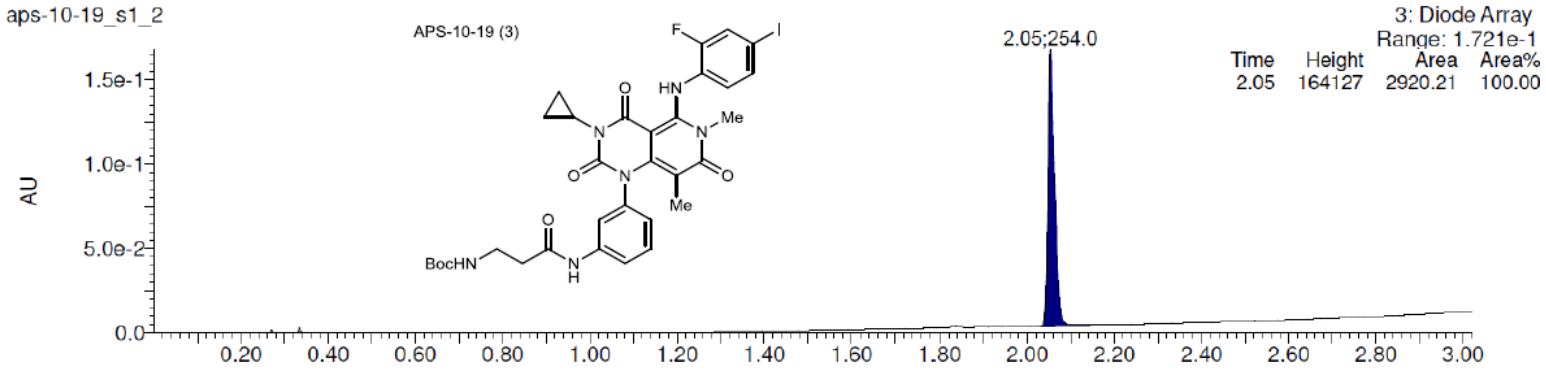
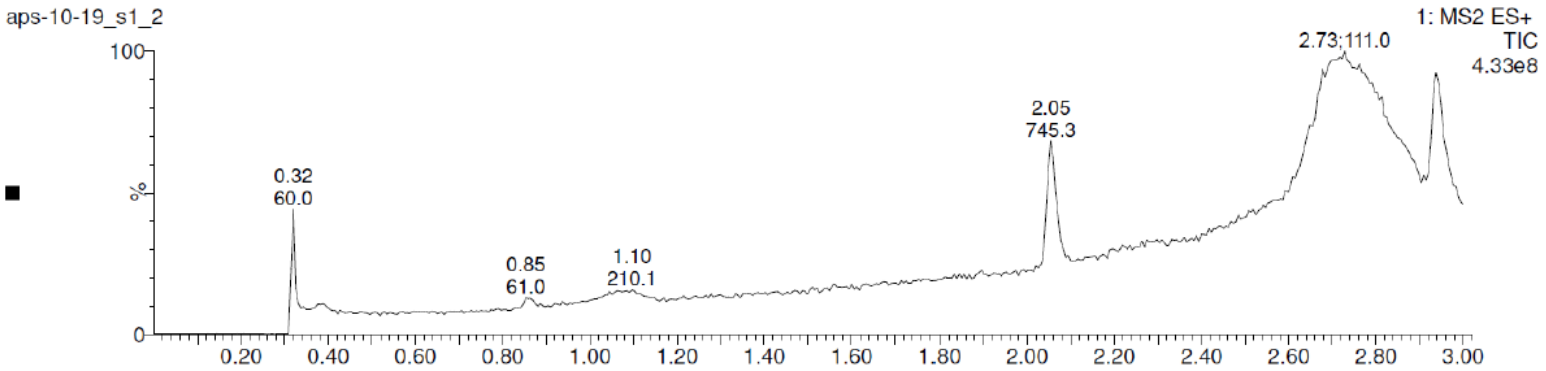
100 200 300 400 500 600 700 800 900 1000

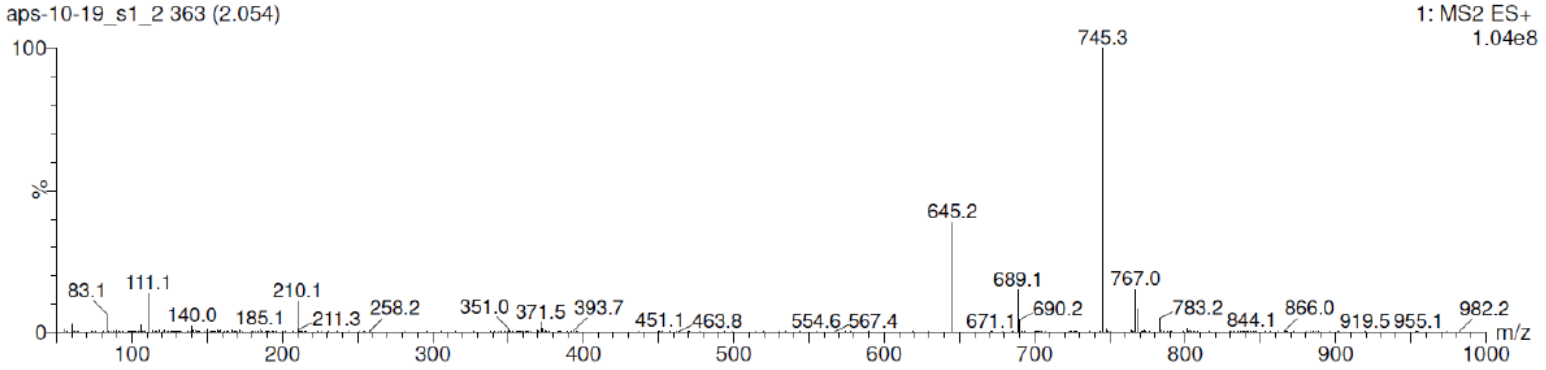

APS-9-20 (2)
F
I
HN
O
N
Me
O
N
O
Me
H_2N
^{1}H NMR 400 MHz
DMSO-d_6
DMSO-d6
11.0636
7.7953
7.7904
7.7648
7.5533
7.5509
7.5295
7.0736
7.0540
7.0345
6.9226
6.9013
6.8793
6.5694
6.5664
6.5462
6.5364
6.5315
6.5266
6.4729
6.4704
6.4533
6.4509
5.2531
water
3.3152
3.0683
2.6296
2.6216
2.6124
2.5000
1.3493
0.9698
0.9520
0.9368
0.6636
0.6440
0.6361
0.6220
1.02
1.09
1.09
1.06
1.07
2.06
1.02
2.11
3.00
1.09
2.95
2.08
2.04
Chemical Shift (ppm)

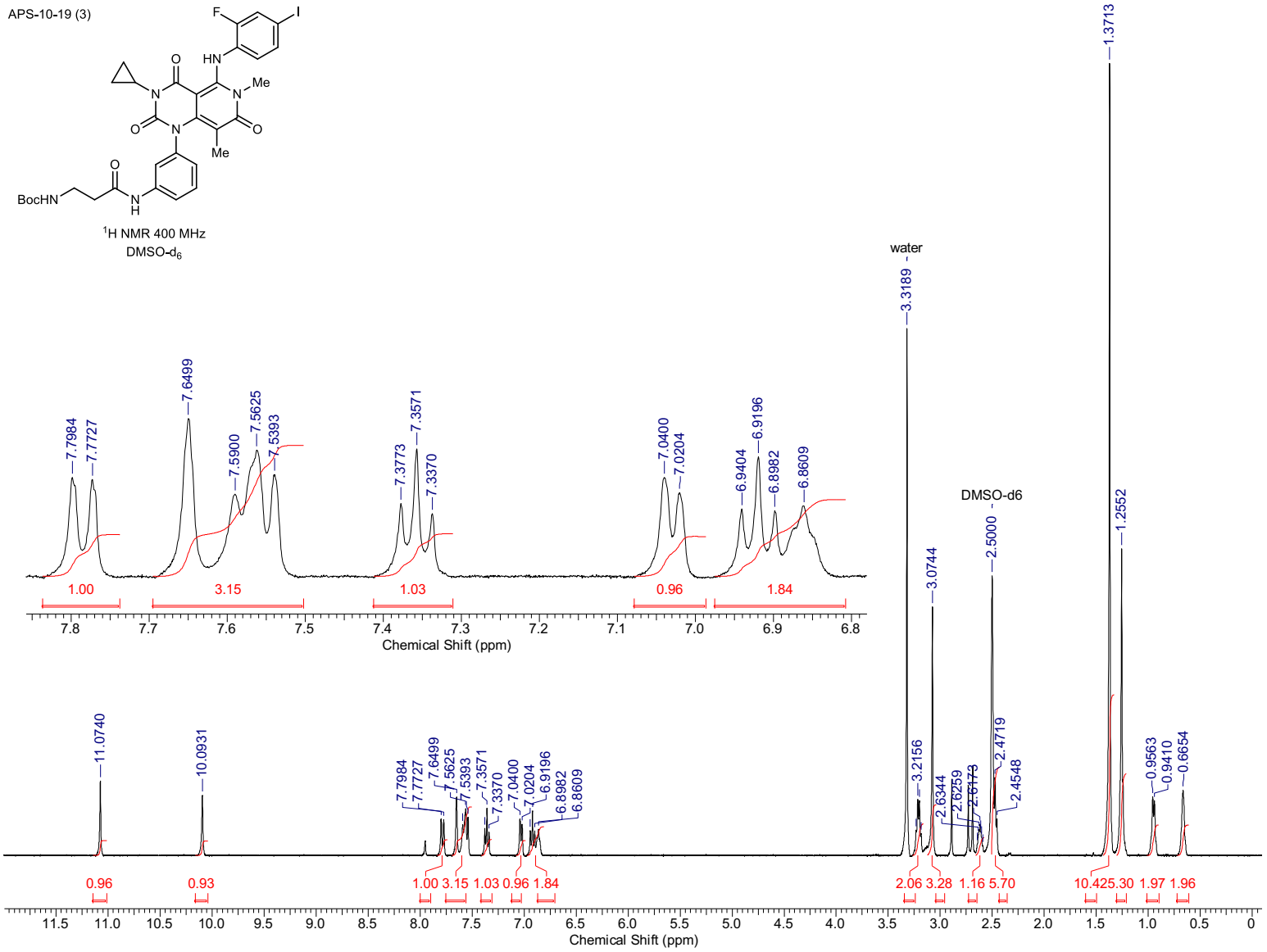

APS-9-20 (2)
CFCL3
0.0000
-123.9432
1.00
^{19}F NMR 376 MHz
DMSO-d_6
Chemical Shift (ppm)

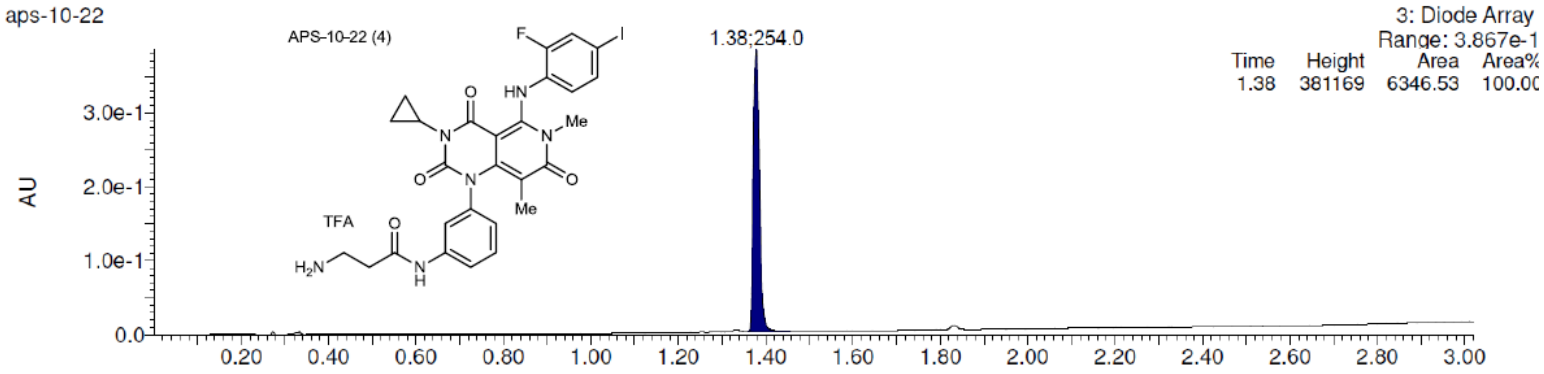
.

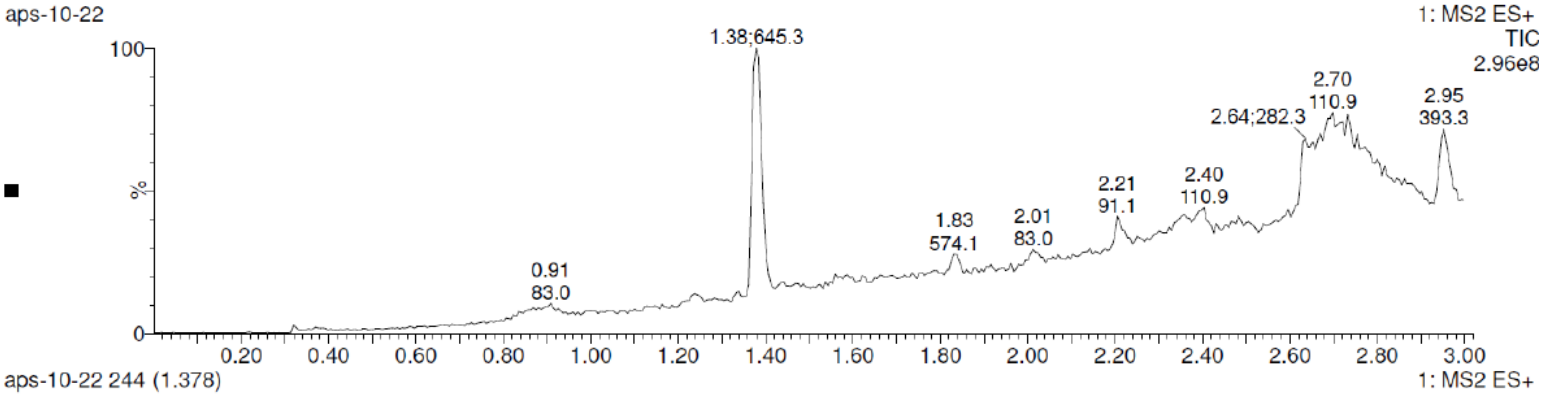

aps-10-19_s1_2
APS-10-19 (3)
3: Diode Array
Range: 1.721e-1
Time Height Area Area%
2.05 164127 2920.21 100.00
2.05;254.0
AU
1.5e-1
1.0e-1
5.0e-2
0.0
0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40 2.60 2.80 3.00

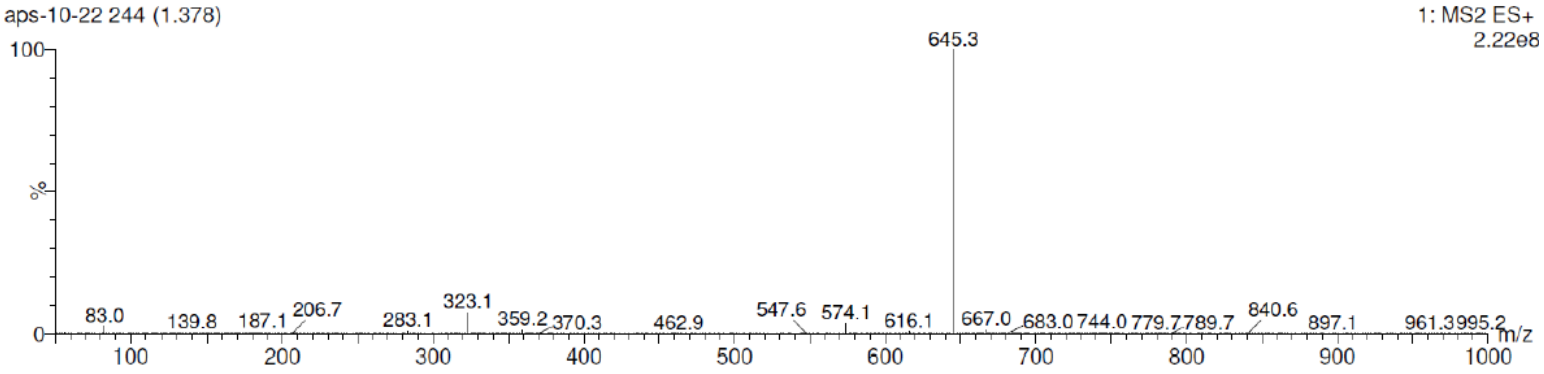

aps-10-19_s1_2
1: MS2 ES+
TIC
4.33e8
2.73;111.0
2.05
745.3
0.32
60.0
0.85
61.0
1.10
210.1
%
100
0
0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40 2.60 2.80 3.00


aps-10-19_s1_2 363 (2.054)
1: MS2 ES+
1.04e8
745.3
645.2
689.1
767.0
83.1
111.1
140.0
185.1
210.1
211.3
258.2
351.0
371.5
393.7
451.1
463.8
554.6
567.4
671.1
690.2
783.2
844.1
866.0
919.5
955.1
982.2
%
100
0
m/z
100 200 300 400 500 600 700 800 900 1000

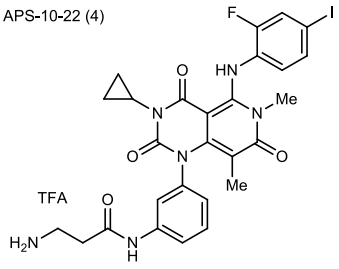

APS-10-19 (3)
F
I
HN
O
N
Me
O
N
O
N
Me
O
O
BocHN
N
H
1H NMR 400 MHz
DMSO-d6
water
DMSO-d6
7.7984
7.7727
7.6499
7.5900
7.5625
7.5393
7.3773
7.3571
7.3370
7.0400
7.0204
6.9404
6.9196
6.8982
6.8609
3.3189
3.0744
2.5000
1.3713
1.2552
11.0740
10.0931
3.2156
2.6344
2.6259
2.4719
2.4548
0.9563
0.9410
0.6654
1.00
3.15
1.03
0.96
1.84
0.93
2.06
3.28
1.16
5.70
10.42
5.30
1.97
1.96
Chemical Shift (ppm)


aps-10-22
APS-10-22 (4)
1.38;254.0
3: Diode Array
Range: 3.867e-1
Time Height Area Area%
1.38 381169 6346.53 100.00
AU
3.0e-1
2.0e-1
1.0e-1
0.0
TFA
0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40 2.60 2.80 3.00

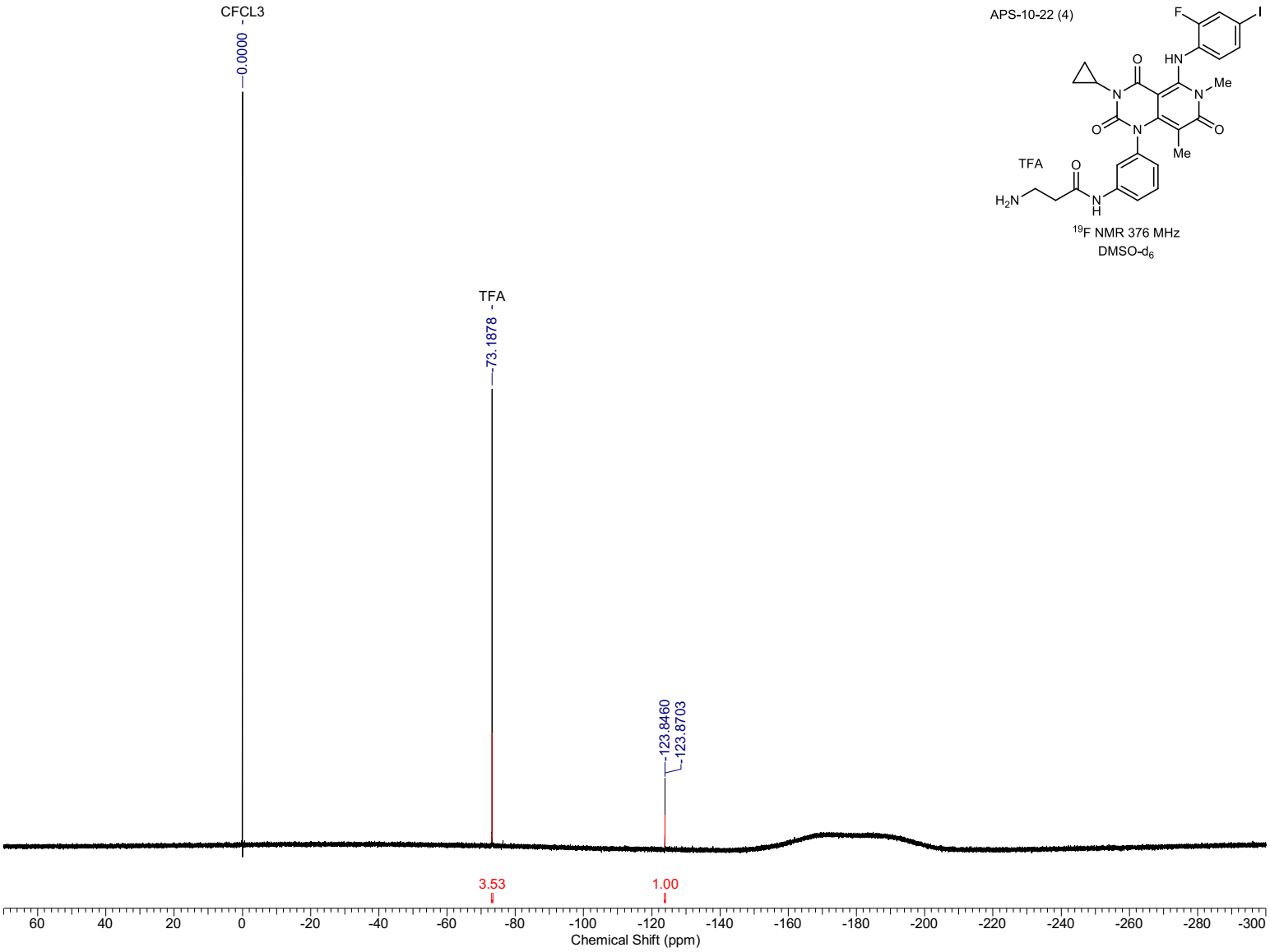

aps-10-22
1: MS2 ES+
TIC
2.96e8
1.38;645.3
2.70
110.9
2.95
393.3
2.64;282.3
2.21
91.1
2.40
110.9
1.83
574.1
2.01
83.0
0.91
83.0
%
100
0
0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40 2.60 2.80 3.00

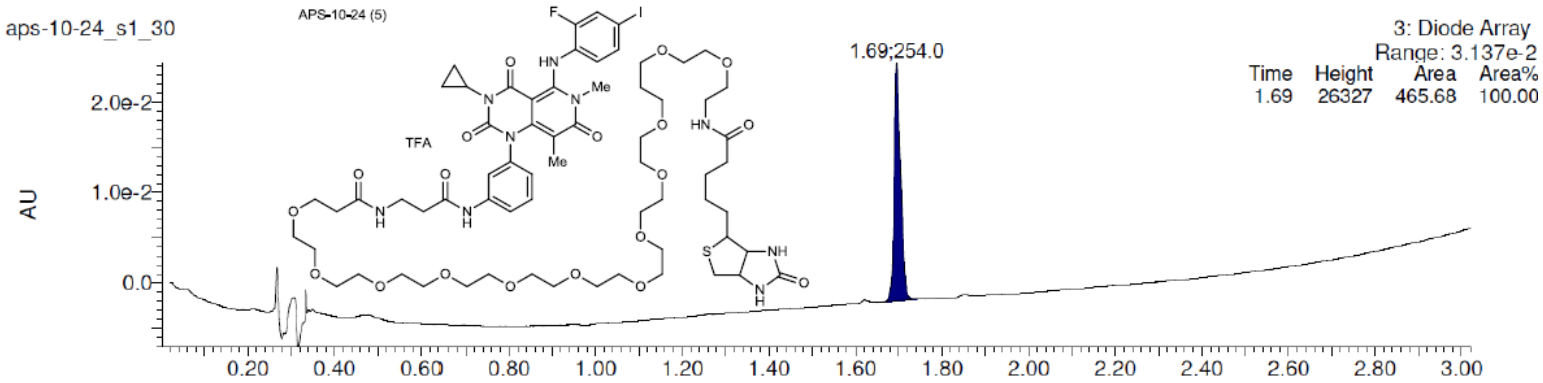

aps-10-22 244 (1.378)
1: MS2 ES+
2.22e8
645.3
100
%
0
83.0
139.8
187.1
206.7
283.1
323.1
359.2
370.3
462.9
547.6
574.1
616.1
667.0
683.0
744.0
779.7
789.7
840.6
897.1
961.3
995.2
m/z
100 200 300 400 500 600 700 800 900 1000

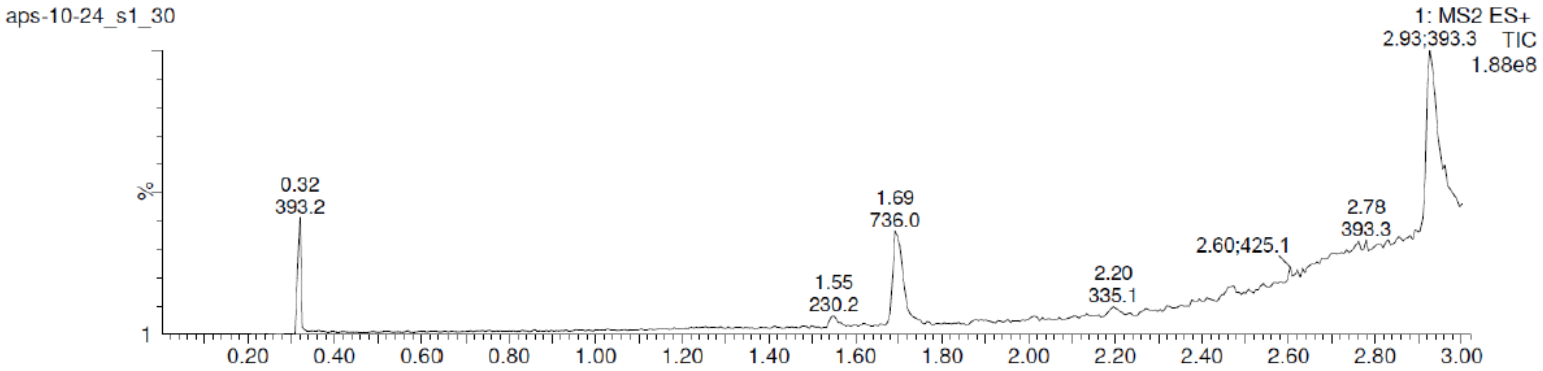
APS-10-22 (4)

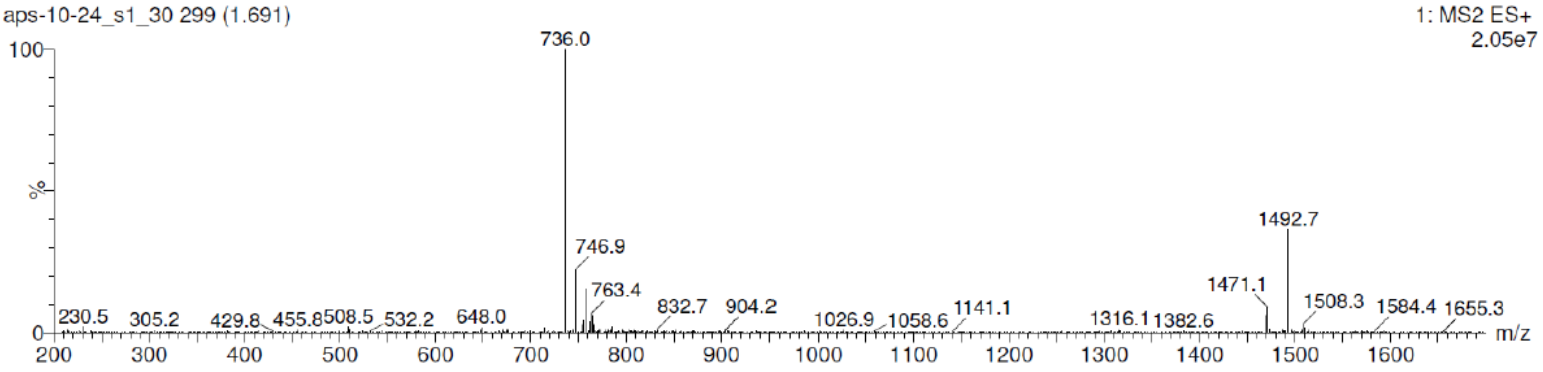


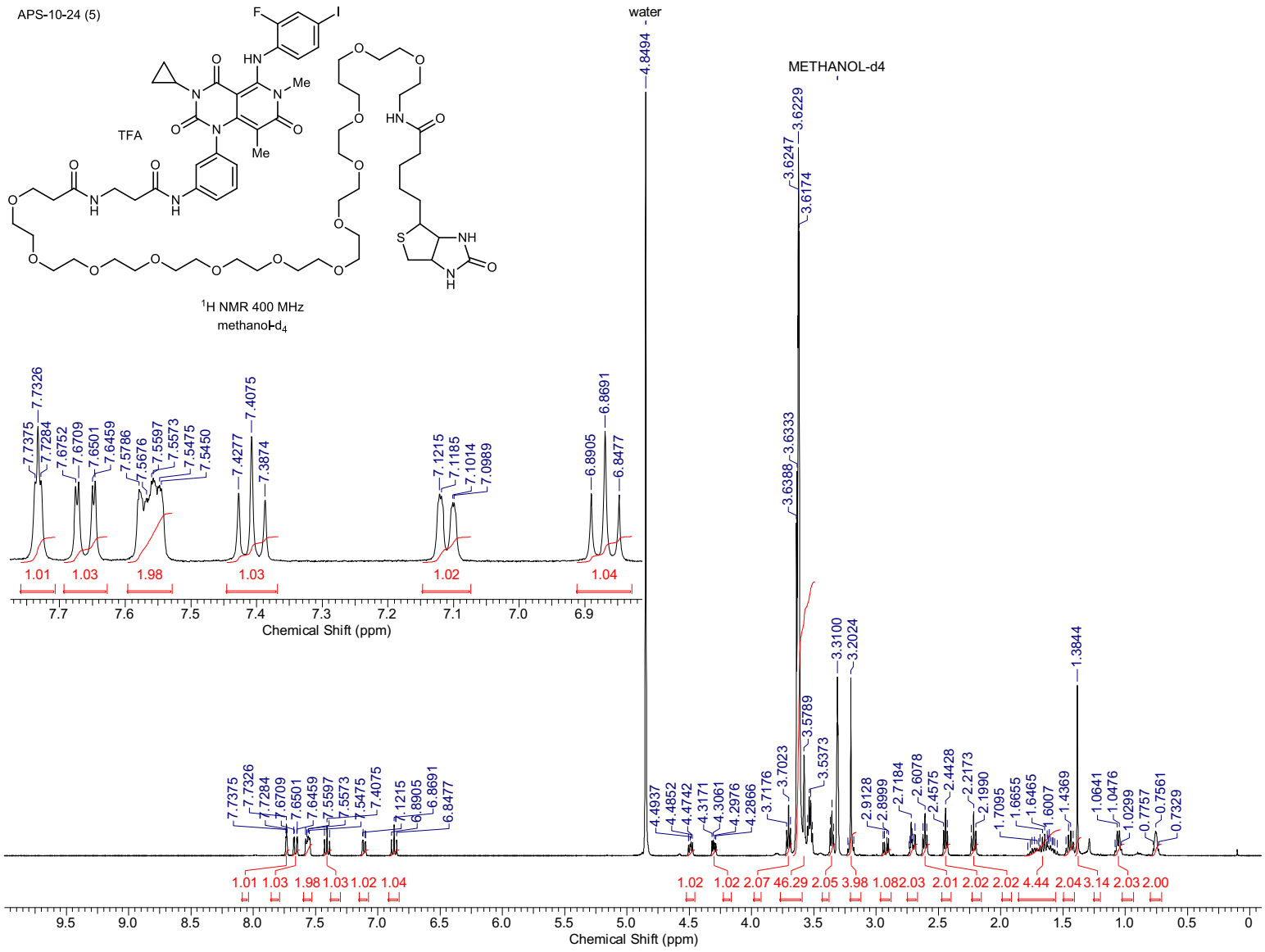
^{1}H NMR 400 MHz
DMSO-d_6

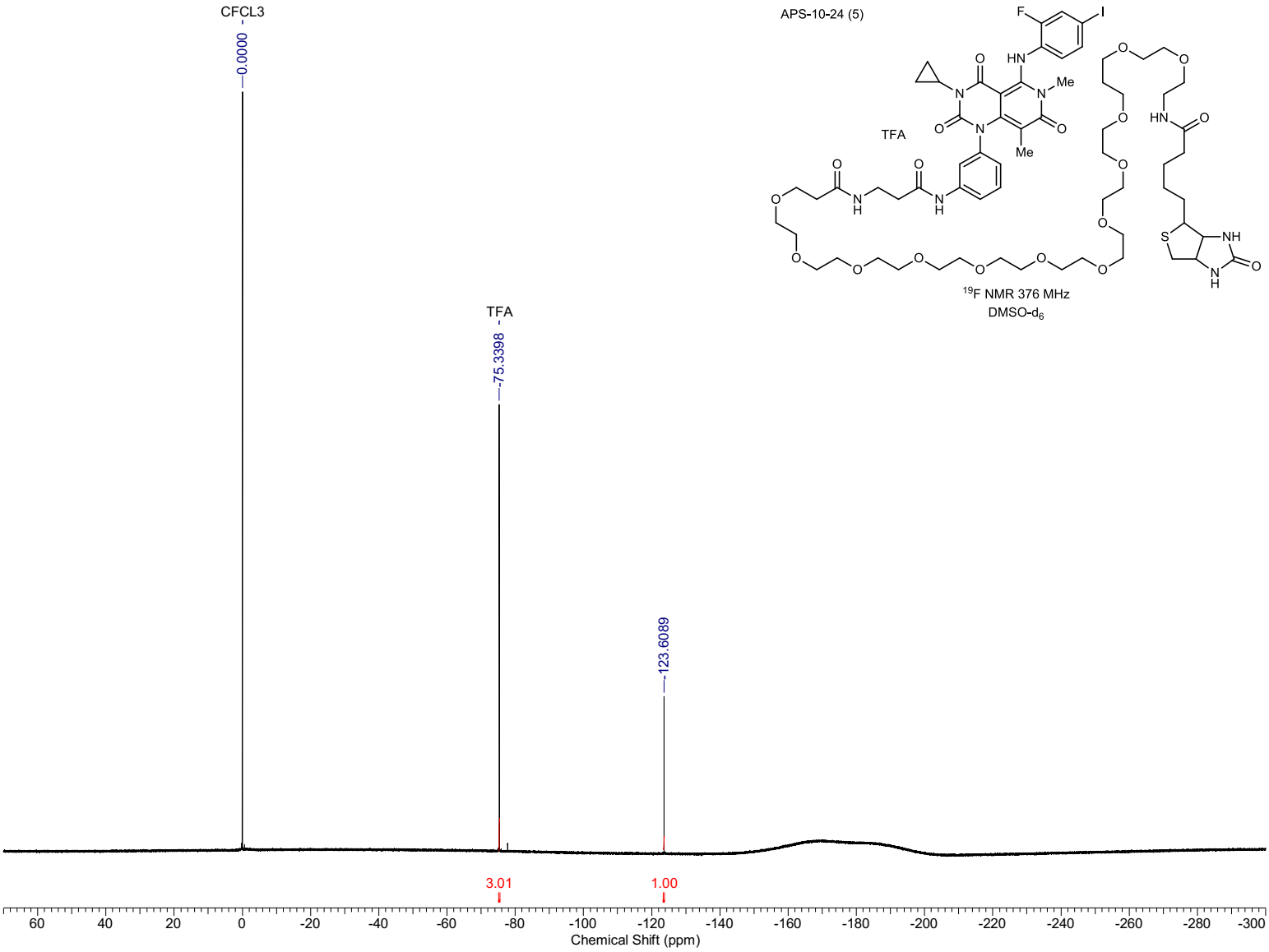

CFCL3
0.0000
TFA
-73.1878
-123.8460
-123.8703
3.53
1.00
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-220
-240
-260
-280
-300
Chemical Shift (ppm)
APS-10-22 (4)
F
I
HN
O
N
Me
O
N
O
N
Me
O
TFA
O
H_2N
N
H
^{19}F NMR 376 MHz
DMSO-d_6

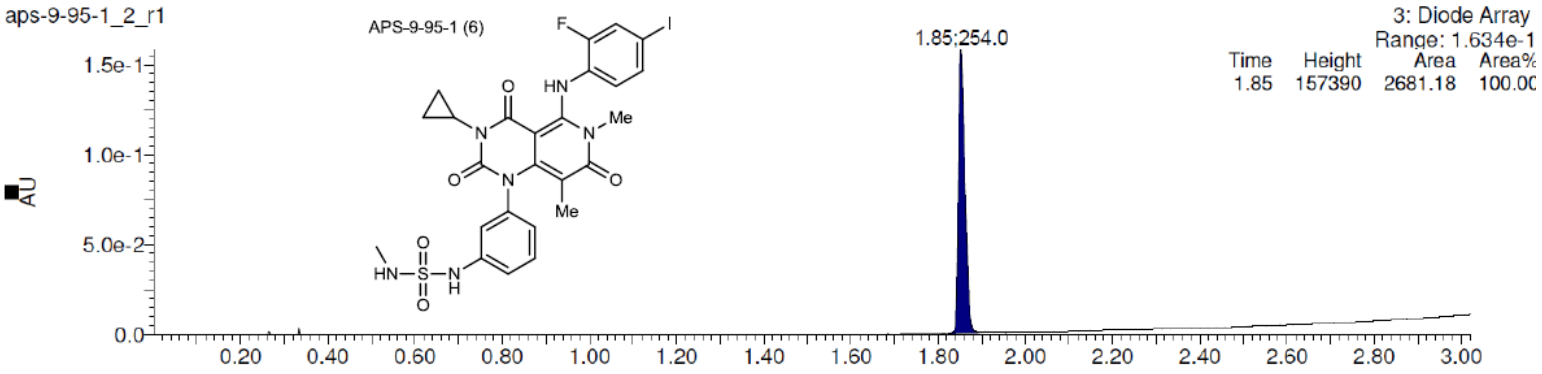

aps-10-24_s1_30
APS-10-24 (5)
TFA
1.69;254.0
3: Diode Array
Range: 3.137e-2
Time Height Area Area%
1.69 26327 465.68 100.00
AU
2.0e-2
1.0e-2
0.0
0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40 2.60 2.80 3.00

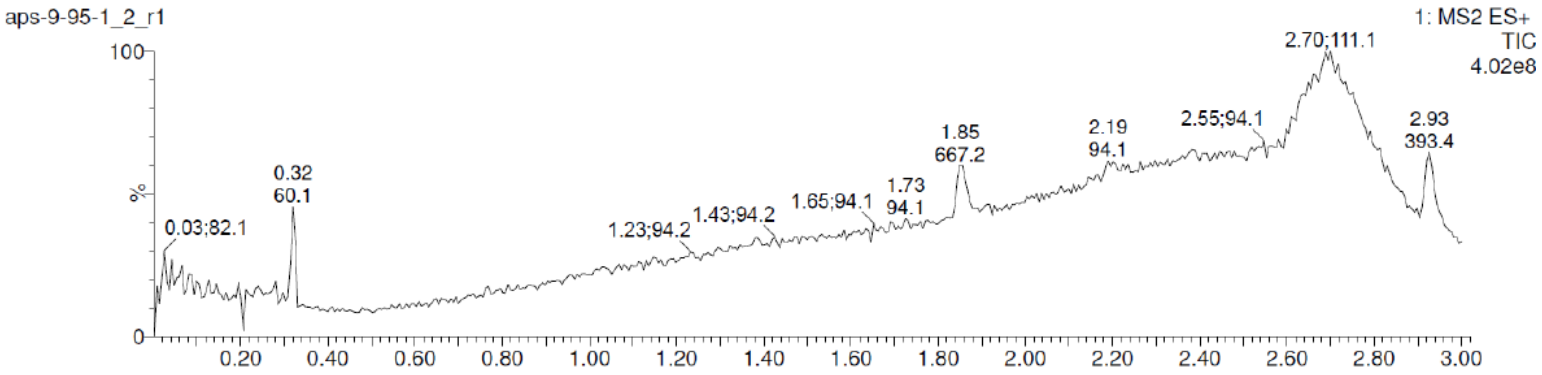

aps-10-24_s1_30
1: MS2 ES+
2.93;393.3 TIC
1.88e8
%
0.32
393.2
1.55
230.2
1.69
736.0
2.20
335.1
2.60;425.1
2.78
393.3
1
0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40 2.60 2.80 3.00

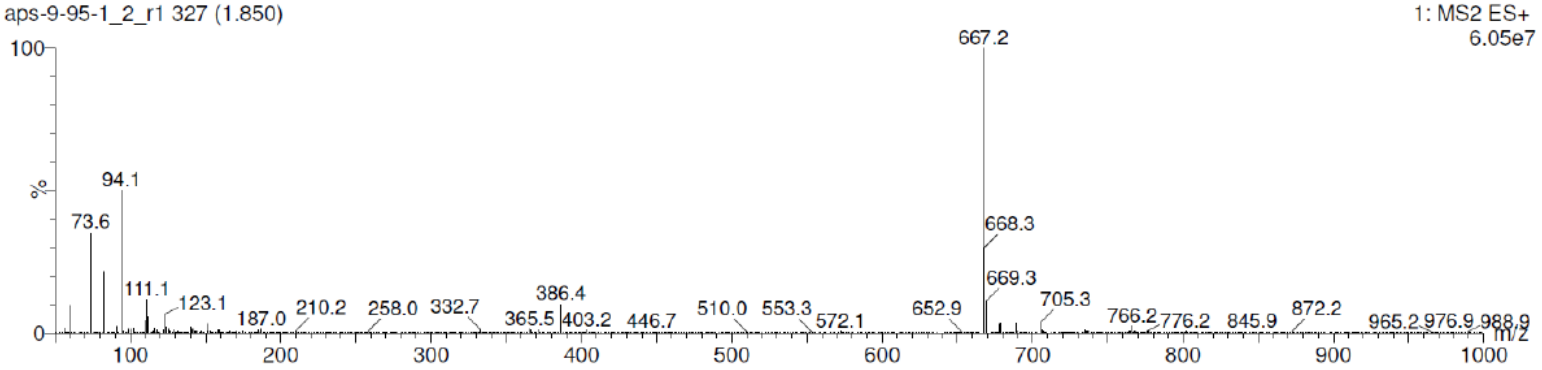

aps-10-24_s1_30 299 (1.691)
1: MS2 ES+
2.05e7
100
%
0
736.0
746.9
763.4
1492.7
1471.1
1508.3
230.5
305.2
429.8
455.8
508.5
532.2
648.0
832.7
904.2
1026.9
1058.6
1141.1
1316.1
1382.6
1584.4
1655.3
m/z
200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600

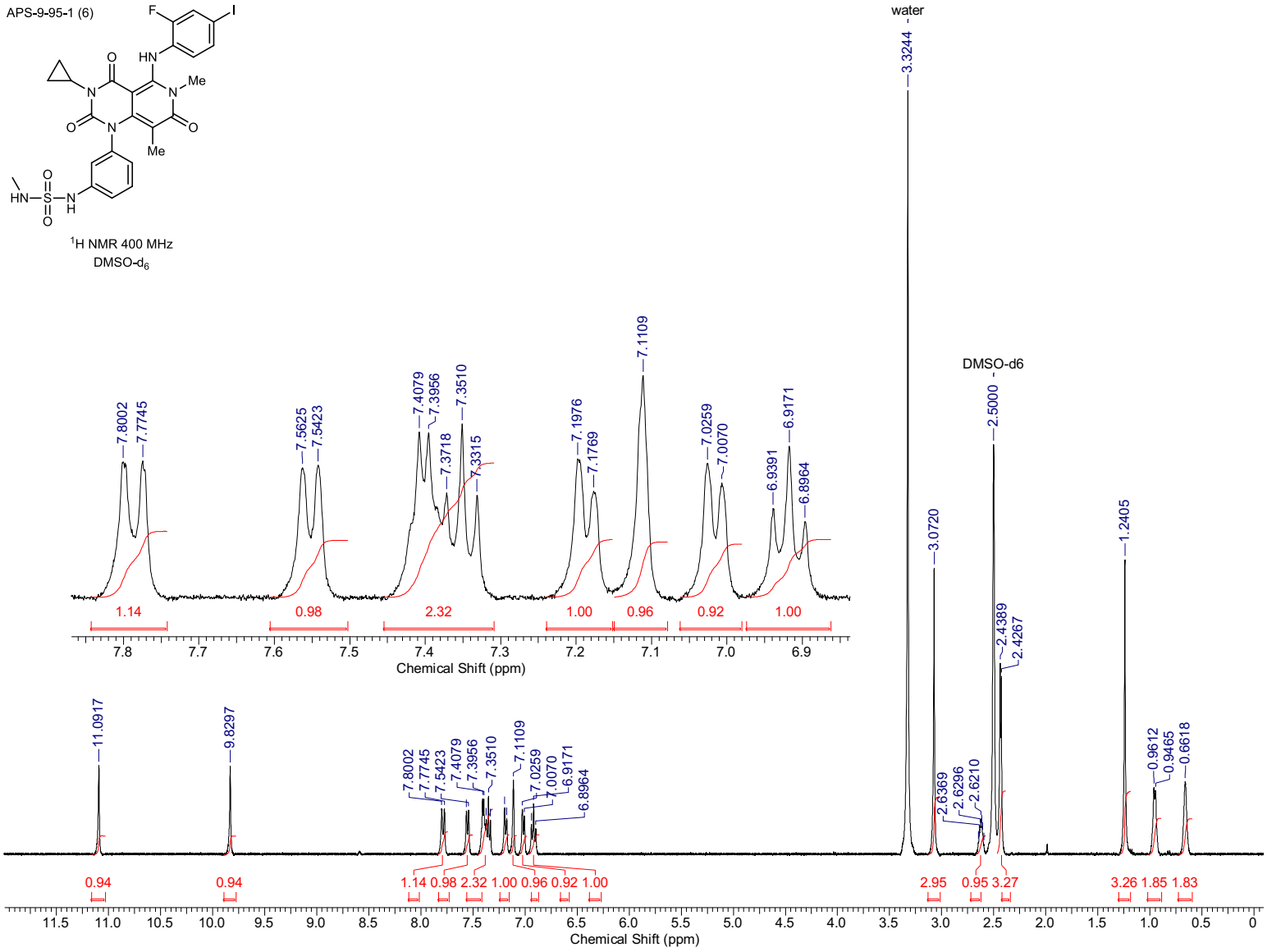

APS-10-24 (5)
TFA
^{1}H NMR 400 MHz
methanol-d$_4$
water
METHANOL-d4
Chemical Shift (ppm)
Chemical Shift (ppm)

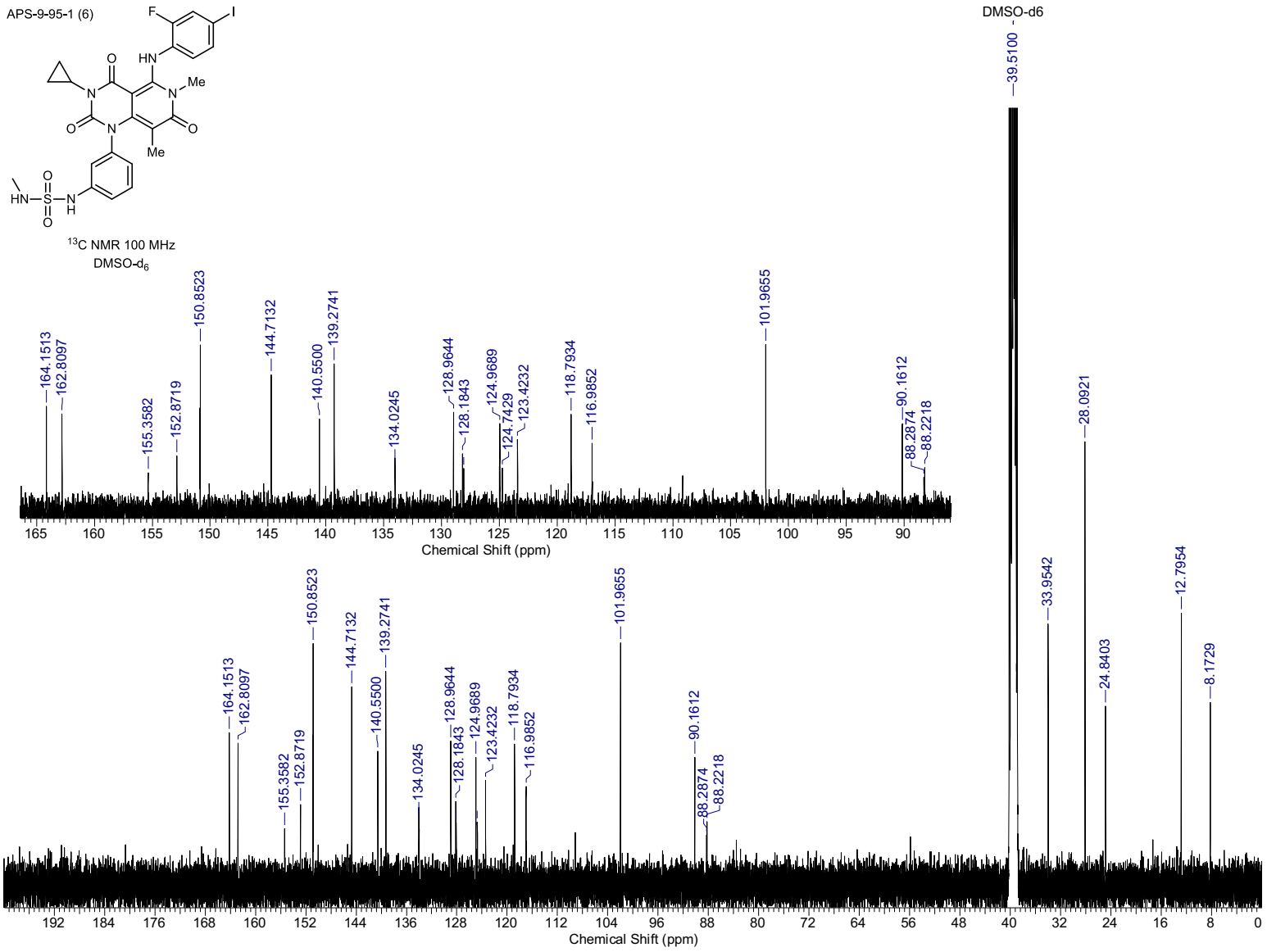

CFCL3
0.0000
TFA
-75.3398
123.6089
3.01
1.00
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-220
-240
-260
-280
-300
Chemical Shift (ppm)
APS-10-24 (5)
TFA
19F NMR 376 MHz
DMSO-d6

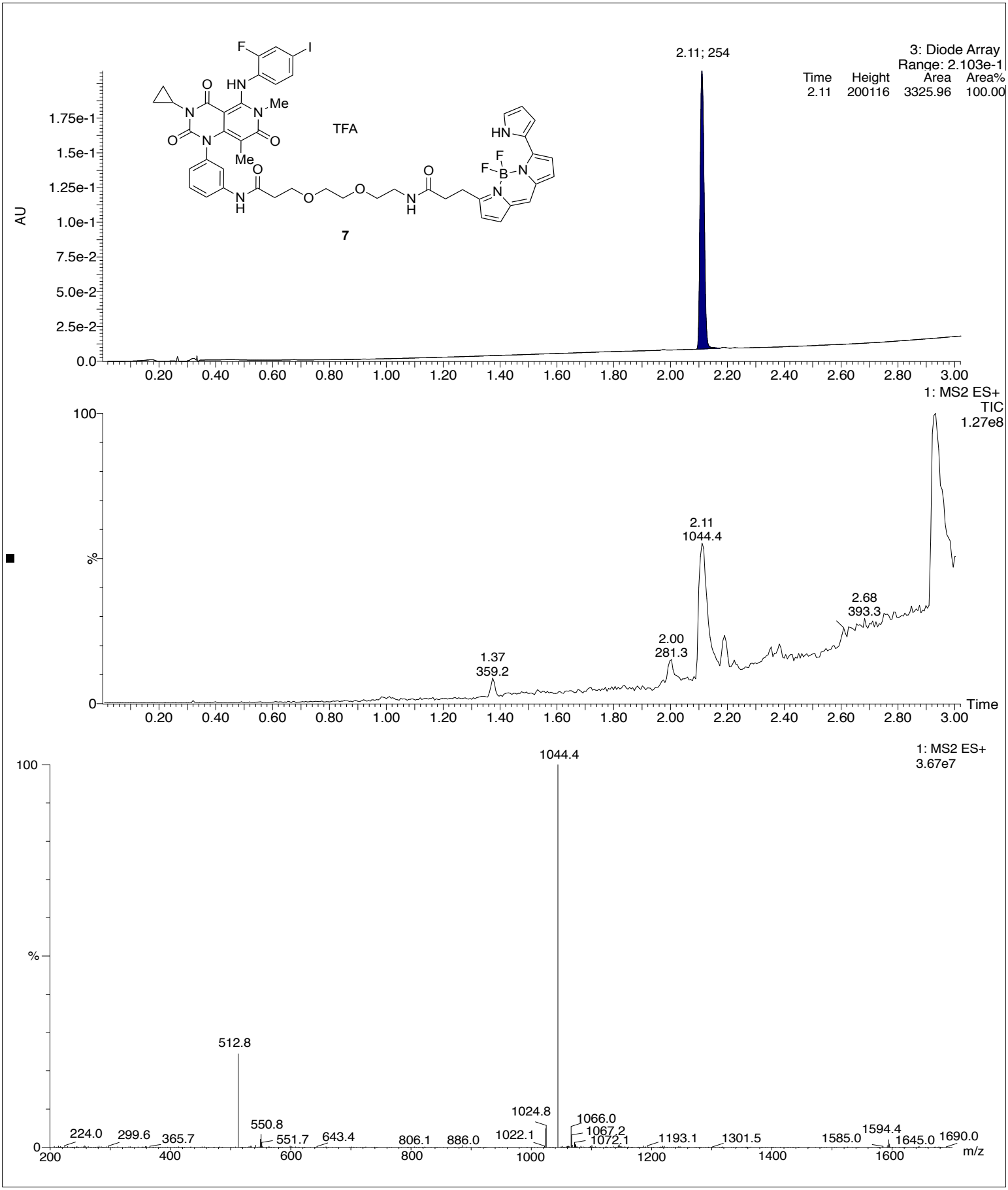

aps-9-95-1_2_r1
APS-9-95-1 (6)
3: Diode Array
Range: 1.634e-1
Time Height Area Area%
1.85 157390 2681.18 100.00
1.85;254.0
AU

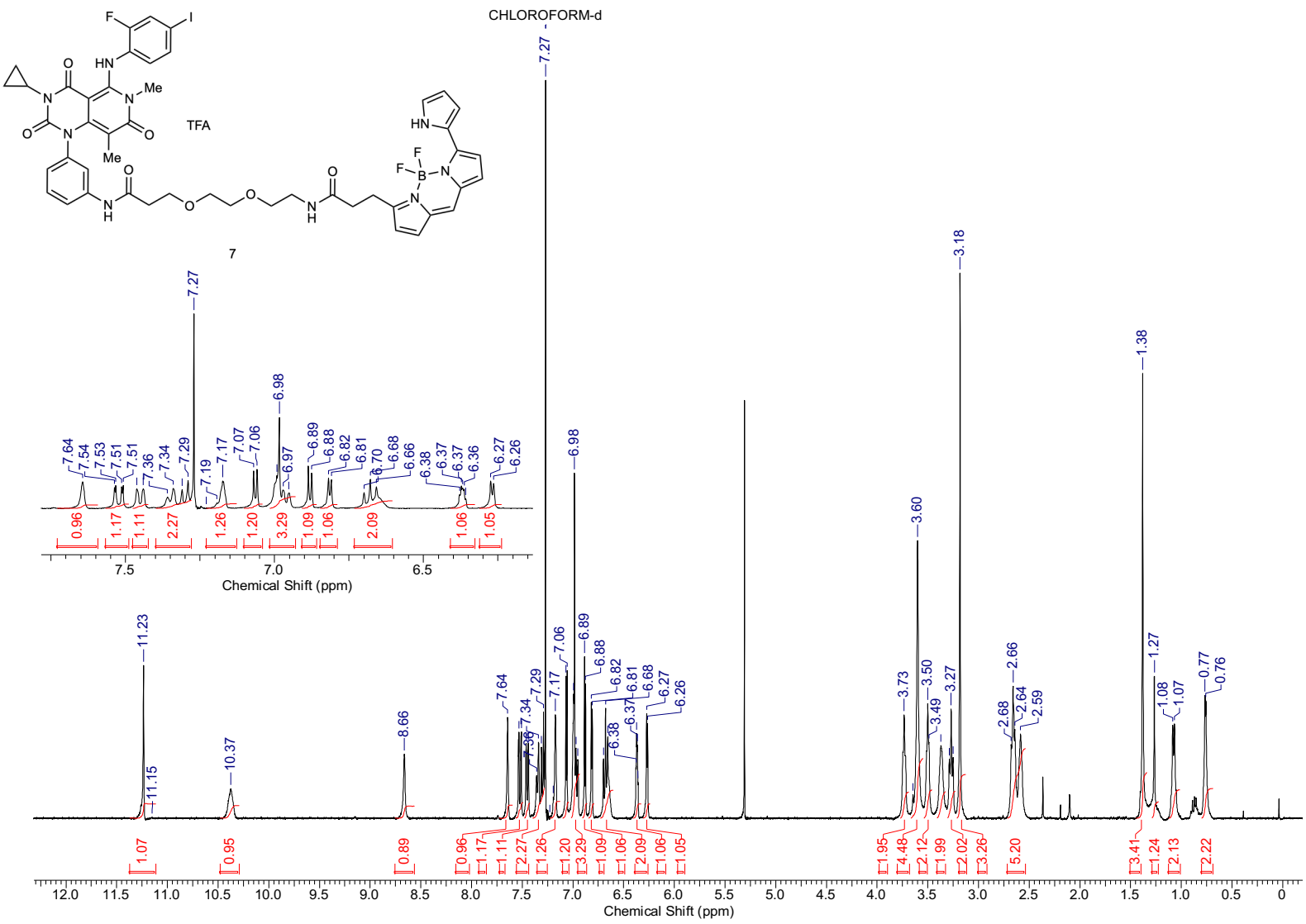

aps-9-95-1_2_r1
1: MS2 ES+
TIC
4.02e8
2.70;111.1
2.55;94.1
2.93
393.4
2.19
94.1
1.85
667.2
1.73
94.1
1.65;94.1
1.43;94.2
1.23;94.2
0.32
60.1
0.03;82.1

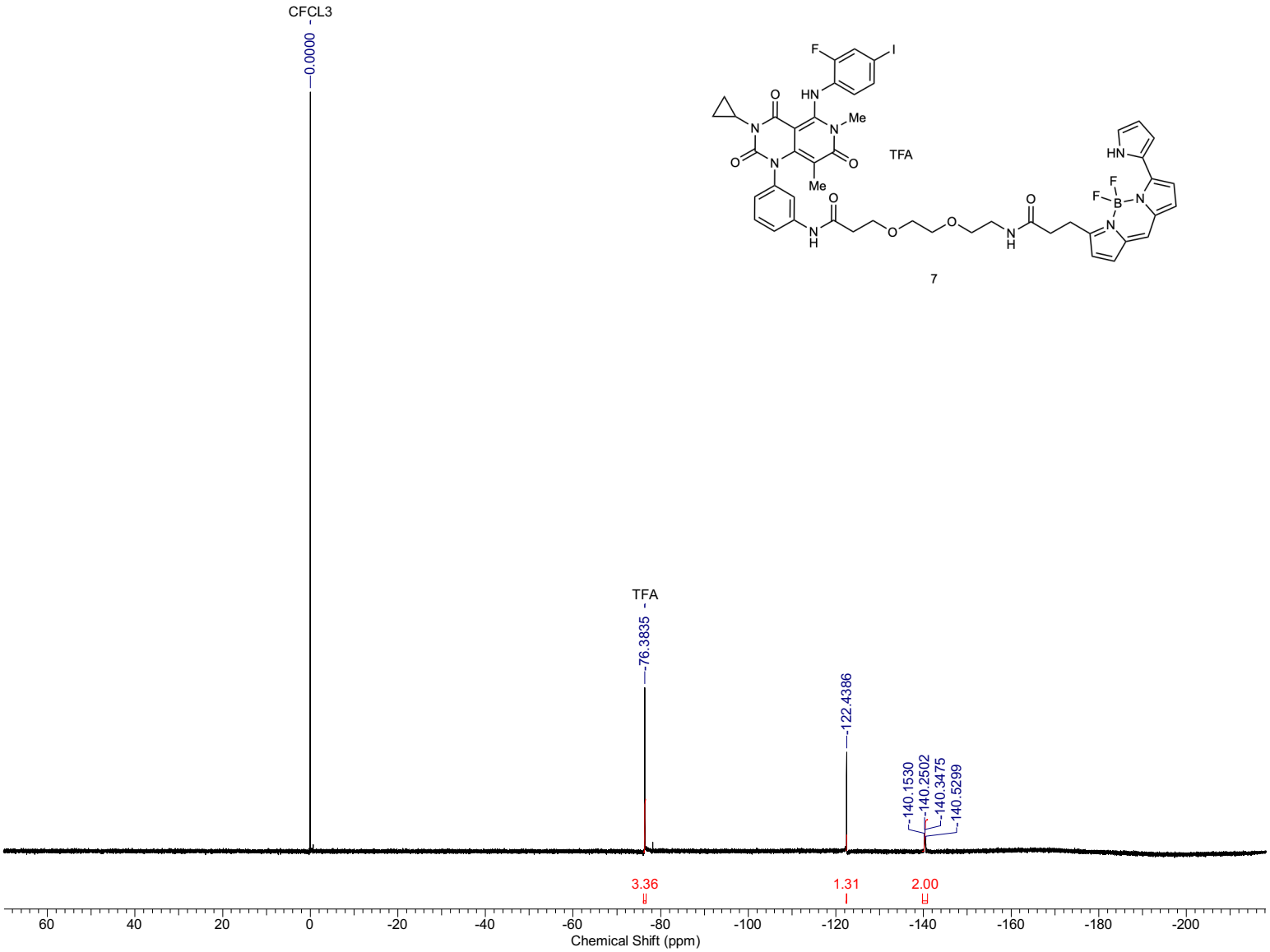

aps-9-95-1_2_r1 327 (1.850)
1: MS2 ES+
6.05e7
667.2
94.1
73.6
668.3
669.3
111.1
123.1
386.4
705.3
187.0
210.2
258.0
332.7
365.5
403.2
446.7
510.0
553.3
572.1
652.9
766.2
776.2
845.9
872.2
965.2
976.9
988.9
m/z


APS-9-95-1 (6)
F
I
HN
O
N
Me
N
O
O
N
Me
O
HN
S
N
H
O
O
1H NMR 400 MHz
DMSO-d6
water
3.3244
DMSO-d6
2.5000
7.8002
7.7745
7.5625
7.5423
7.4079
7.3956
7.3718
7.3510
7.3315
7.1976
7.1769
7.1109
7.0259
7.0070
6.9391
6.9171
6.8964
3.0720
2.4389
2.4267
1.2405
11.0917
9.8297
2.6369
2.6296
2.6210
0.9612
0.9465
0.6618
1.14
0.98
2.32
1.00
0.96
0.92
1.00
0.94
0.94
2.95
0.95
3.27
3.26
1.85
1.83
Chemical Shift (ppm)
Chemical Shift (ppm)


APS-9-95-1 (6)
DMSO-d6
39.5100
^{13}C NMR 100 MHz
DMSO-d_6
164.1513
162.8097
155.3582
152.8719
150.8523
144.7132
140.5500
139.2741
134.0245
128.9644
128.1843
124.9689
124.7429
123.4232
118.7934
116.9852
101.9655
90.1612
88.2874
88.2218
28.0921
33.9542
24.8403
12.7954
8.1729
Chemical Shift (ppm)


F
I
HN
O
N
Me
O
N
Me
O
TFA
HN
F
F
B
N
N
O
O
O
O
N
H
N
H
7
2.11; 254
3: Diode Array
Range: 2.103e-1
Time Height Area Area%
2.11 200116 3325.96 100.00
AU
1.75e-1
1.5e-1
1.25e-1
1.0e-1
7.5e-2
5.0e-2
2.5e-2
0.0
0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40 2.60 2.80 3.00
1: MS2 ES+
TIC
1.27e8
100
%
2.11
1044.4
2.68
393.3
2.00
281.3
1.37
359.2
0
Time
0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40 2.60 2.80 3.00
1: MS2 ES+
3.67e7
1044.4
100
%
512.8
1024.8
1066.0
1067.2
550.8
224.0
299.6
365.7
551.7
643.4
806.1
886.0
1022.1
1072.1
1193.1
1301.5
1585.0
1594.4
1645.0
1690.0
0
200 400 600 800 1000 1200 1400 1600
m/z


CHLOROFORM-d
TFA
7
Chemical Shift (ppm)
Chemical Shift (ppm)


CFCL3
0.0000
TFA
76.3835
122.4386
140.1530
140.2502
140.3475
140.5299
3.36
1.31
2.00
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
Chemical Shift (ppm)
TFA
7