Reducing Limitation in Probe Design: The Development of a Diazirine-Compatible Suzuki-Miyaura Cross Coupling Reaction

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Supporting information

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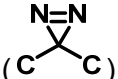
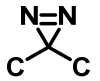
I. Literature Survey

We used multiple search strategies to identify diazirine PAL probes and their syntheses, since no one method identified all relevant papers. Specifically, the use of substructure searching alone proved insufficient since PAL studies are commonly published without chemical structures in the article text, and literature database indexing of structures in journal supporting information sections is inconsistent. We found that a combination of substructure and text searches provided sufficient coverage for our analysis.

The following searches were conducted:

1. ChEMBL was searched using a diazirine substructure () and returned 134 compounds in 55 papers.

2. ThomsonPharma was searched using a diazirine substructure () and returned 209 compounds in 111 papers.

3. PubMed was searched using the term “photoaffinity” and papers filtered to a limited set of journals in which PAL studies frequently appear (Angew Chem Int Ed Engl, J Am Chem Soc, Nat Chem Biol, Nat Commun, Nat Struct Mol Biol, Proc Natl Acad Sci USA), returning 203 papers.

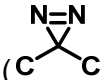
4. Scifinder was searched using the 3 strategies below – results were pooled.

    a. Get references from a recent PAL study

        i. search by Document Identifier with this DOI: http://dx.doi.org/10.1016/j.cell.2016.12.029

        ii. Get Cited references (54 refs)

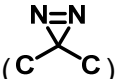
        iii. Get Substances from the Cited References (859 substances)

        iv. Refine by substructure ( , no locked rings or atoms) (119 substances)

        v. Get References with this subset of 119 substances, limited to Reactant/Reagent or Biological Study (859 substances)

        vi. Get References (74)

    b. Get references based on substructure search

        i. Diazirine structure () search (3719 substances)

ii. Get references (1648 after removing duplicates)

iii. Analyze by Index Term (Show more, select Photoaffinity, Photoaffinity labeling, Photocrosslinking) = 258 references

c. Explore by Research Topic

i. Search text “photoaffinity of diazirines in proteomics studies”

ii. 39 references with all three concepts present anywhere

References from search #1, which span 1990-2016, were analyzed as follows:

1. Diazirine-containing probes used in PAL studies were identified
2. Probes were categorized as Nested, Replacement, or Appended as defined by Hill and Robertson.[1] The authors focus on trifluoromethyl phenyl diazirine probes, but for the analysis conducted here the definitions were applied to aliphatic diazirines as well.
3. Diazirine synthons used for the synthesis of these probes were identified
4. The reaction used to incorporate the diazirine into the probe was categorized
5. Other reactions performed on diazirine-containing intermediates were listed

References from searches #2, #3, and #4 were combined, and duplicates and patent references were removed, resulting in 655 references. To sample recent literature, references prior to 2012 were filtered out to leave 161 papers, which were analyzed as follows:

1. Papers lacking any diazirine containing compounds were removed
2. Diazirine-containing probes used in PAL studies were identified
3. Probes were categorized as Nested, Replacement, or Appended as defined by Hill and Robertson.[1] The authors focus on trifluoromethyl phenyl diazirine probes, but for the analysis conducted here the definitions were applied to aliphatic diazirines as well.
4. Diazirine synthons used for the synthesis of these probes were identified
5. The reaction used to incorporate the diazirine into the probe was categorized
6. Other reactions performed on diazirine-containing intermediates were listed

The combined analyses are presented in Figure 1 in the text. For full literature search results see Supporting Information table attached as a Microsoft Excel file.

II. PDB Search of Biaryl Ligands in Protein Binding Sites

On March 22, 2018, a file with SMILES[2] representations for all PDB ligands was downloaded from https://www.rcsb.org. Pipeline Pilot (http://www.accelrys.com) protocols were used to remove molecules with a molecular weight > 600 Da, reducing the original set of 23,469 molecules to 21,280. A SMARTS[3] query was then applied to identify all small molecules containing a biaryl moiety. Overall, 3,791 small molecules matched the query at least one time. These molecules mapped to 4,522 crystal structures in the PDB – the number of crystal structures was larger than the number of ligands because some small molecules occurred in multiple PDB entries. All 4,522 crystal structures were downloaded from the PDB. An in-house written python script using the OpenEye (http://www.eyesopen.com) chemistry toolkit was then used to measure the shortest distance between any atom in the biaryl moiety and any heavy atom in the protein. Please note that this shortest distance could only be measured for 4,186 PDB entries. For 336 PDB entries, no biaryl moiety could be identified in the co-crystallized small molecule using the same SMARTS query that was applied in the Pipeline Pilot protocols. This is likely due to differences in the perception of SMARTS and aromaticity by different chemical software tools. For 4,107 of the 4,186 PDB entries (98%), the shortest distance between any biaryl atom and any heavy protein atom was ≤ 5 Angstrom.

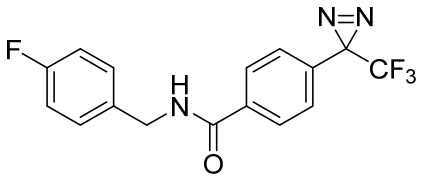
III. General Experimental Materials and Methods

Unless otherwise noted, all reactions were conducted under inert atmosphere (N_2). Reactions were monitored using a Waters Aquity UPLC system with UV detection at 220, 254 or 280 nm and a low resonance electrospray mode (ESI).

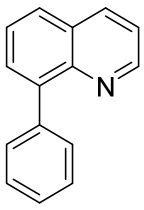
All NMR spectra were recorded on a Bruker spectrometer (500 MHz) at ambient temperature. ^{1}H NMR chemical shifts are reported as δ in units of parts per million (ppm) relative to methanol-d_4 (δ 3.31, quintet) or dimethylsulfoxide-d_6 (δ 2.50, quintet). Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), br (broad) or m (multiplet). Coupling constants are reported as a *J* value in Hertz (Hz). The number of protons (*n*) for a given resonance is indicated as *n*H and is based on spectral integration values. ^{13}C NMR chemical shifts are reported as δ in units of parts per million (ppm) relative to methanol-d_4 (δ 49.2, septet) or dimethylsulfoxide-d_6 (δ 39.5, septet). ^{19}FNMR chemical shifts are reported as δ in units of parts per million (ppm). High resolution mass spectra (HRMS) were recorded using a Waters Synapt G1 Quadrapole-Time of Flight (Q-TOF) high definition mass spectrometer (HDMS).

Unless otherwise noted all materials were obtained from commercial suppliers and used without further purification. Anhydrous organic solvents were purchased from Sigma Aldrich in SureSeal™ bottles. Concentrated acetic acid was purchased from Sigma Aldrich and degassed for further use. Xphos G2, K_3PO_4 tribasic, triethylamine, 4-fluorobenzylamine, phenylboronic acid, phenylboronic acid pinacol ester, 8-bromoquinoline, 8-chloroquinoine and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate were obtained from Aldrich. 4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoic acid, *t*BuOCl and *t*BuOH were purchased from TCI America.

IV. Procedures for Preparation of Starting Materials and Product Standards for Suzuki-Miyaura Coupling Reactions

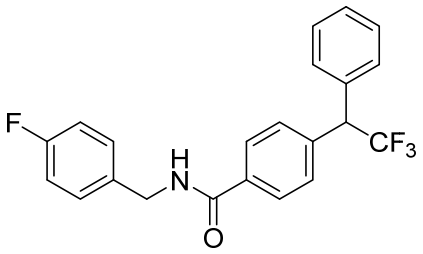


N-(4-fluorobenzyl)-4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzamide (1): 4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoic acid (800 mg, 3.48 mmol) was dissolved in anhydrous DMF (30 mL) under inert atomsphere. Triethylamine (0.969 ml, 6.95 mmol) followed by (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (2152 mg, 4.87 mmol) were added to the stirring solution and the resulting mixture was allowed to stir for 10min. 4-fluorobenzylamine (522 mg, 4.17 mmol) was then added via syringe and the resulting solution was allowed to stir for 16h. The solution was diluted with EtOAc (200 mL), washed with aqueous saturated brine solution (x2), dried over MgSO4, filtered and concentrated *in vacuo*. The crude product was purified using silica gel chromatography (80g silica gel cartridge, 0-70% EtOAc in hexane over 30 minutes). Pure fractions were combined and concentrated *in vacuo* to yield a white solid (1055 mg, 90% yield). ^{1}H NMR (700 MHz, CDCl3) δ 7.79 (d, *J* = 8.4 Hz, 2H), 7.27 (dd, *J* = 8.3, 5.4 Hz, 2H), 7.21 (d, *J* = 8.3 Hz, 2H), 7.00 (t, *J* = 8.6 Hz, 2H), 4.56 (d, *J* = 5.8 Hz, 2H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 166.4, 163.1 (d, *J* = 225 Hz), 135.3, 133.7, 132.6, 129.6, 127.5, 126.6, 122.1 (q, *J* = 285 Hz), 115.7, 43.6, 28.56 (q, *J* = 41 Hz). HRMS (ESI) $[M+H]^+$ calculated for $C_{16}H_{12}F_4N_3O^+$ 338.0911, found 338.0892.

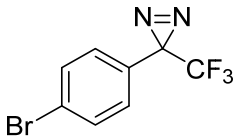


8-phenylquinoline (6) was prepared according to a literature procedure for use as a product standard.[4] To a dry and clean 100 mL three-necked flask containing a magnetic stir bar were charged 8-bromoquinoline (500 mg, 2.4 mmol, 1.0 equiv), pinacol bis(pinacolato)diboron (732mg, 2.9 mmol, 1.2 equiv), $Pd_2(dba)_3$ (22 mg, 0.024 mmol, 1 mol%), *n*-BuPAd$_2$ (26mg, 0.072 mmol, 3 mol %), KOAc (710mg, 7.2 mmol, 3 equiv), and DMAc (24 mL) under nitrogen. Then the reaction mixture was heated to 90 °C and stirred for 2h. After 2h, degassed aqueous K_2CO_3 (4 M, 2.4 mL, 9.6 mmol, 4 equiv) and phenyl bromide (453mg, 2.9 mmol, 1.2 equiv) were charged. The resulting mixture was heated at 90 ºC for 2h. After the reaction mixture was cooled to room temperature, water (100 mL) and EtOAc (100 mL) were added. The aqueous layer was extracted with EtOAc (3 x 80 mL). The combined organic layers were washed with water (100 mL) and brine (100 mL), dried over anhydrous sodium sulfate, and concentrated. Purification of the crude product by column chromatography on silica gel afforded 127mg of 8-phenylbromoquinoline **6** as a yellow oil in 26% yield. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.02 (dd, *J* = 4.2, 1.8 Hz, 1H), 8.24 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.87 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.81 – 7.74 (m, 3H), 7.65 (dd, *J* = 8.0, 7.2 Hz, 1H), 7.60 – 7.51 (m, 2H), 7.50 – 7.42 (m, 2H). ^{13}C NMR (126 MHz,

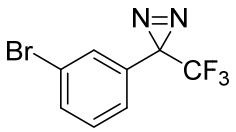
Chloroform-*d*) δ 150.21, 145.91, 140.89, 139.50, 136.43, 130.64, 130.43, 128.94, 128.05, 127.56, 127.43, 126.34, 121.00. HRMS (ESI) $[M+H]^+$ calculated for $C_{15}H_{12}N^+$ 206.0964, found 206.0971.



N-(4-fluorobenzyl)-4-(2,2,2-trifluoro-1-phenylethyl)benzamide (7): To a nitrogen-filled glovebox, phenyl boronic acid (25mg, 0.2 mmol, 1.0 equiv), Sphos Pd G2 (7.2 mg, 10µmol, 5 mol%), and diazirine **1** are added to 8 mL vial with a magnetic stir bar. The vial was brought in the glove box and the aq 1.5 M K_3PO_4 was added followed by THF (2 mL, 0.1M) and stirred at 40ºC overnight. After the reaction is cooled down, EtOAc (5 mL) was added and washed with 10mL of H_2O. The aqueous layer was extracted 3 times with EtOAc and then the combined organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated in vacuo and the crude mixture was purified using flash column chromatography (SiO_2 gel, 30% isocratic EtOAc/hexane for 10 minutes, then 50-100% EtOAc/hexane over 7 minutes) to afford 70 mg of white solid **7** in 90% yield. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.78 (d, *J* = 8.3 Hz, 2H), 7.44 (d, *J* = 8.2 Hz, 2H), 7.39 – 7.34 (m, 4H), 7.30 (dd, *J* = 8.2, 5.3 Hz, 2H), 7.02 (t, *J* = 8.6 Hz, 2H), 6.70 (s, 1H), 4.59 (m, 2H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 166.80, 162.29 (d, *J* = 246.1 Hz), 139.09 (d, *J* = 1.6 Hz), 134.68 (d, *J* = 1.6 Hz), 133.87 (d, *J* = 3.3 Hz), 133.84, 129.57 (d, *J* = 8.2 Hz), 129.41 (q, *J* = 1.2 Hz), 129.06 (q, *J* = 1.2 Hz), 128.87, 128.22, 127.38, 125.89 (q, *J* = 280.6 Hz), 115.66 (d, *J* = 21.5 Hz), 55.31 (q, *J* = 27.8 Hz), 43.42. ^{19}F NMR (471 MHz, Chloroform-*d*) δ -65.72, -114.86. HRMS (ESI) $[M+H]^+$ calculated for $C_{22}H_{18}F_4NO^+$ 388.1319, found 388.1316.

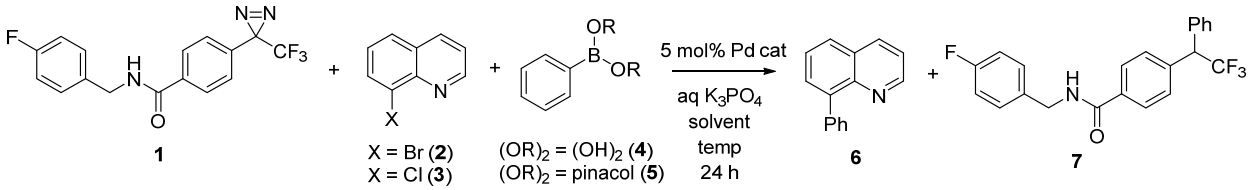


3-(4-bromophenyl)-3-(trifluoromethyl)-3H-diazirine (8): This compound was synthesized according to the literature procedure and NMR spectroscopic information for **8** was identical to those reported in the literature.[5]



3-(3-bromophenyl)-3-(trifluoromethyl)-3H-diazirine (33): This compound was synthesized according to the literature procedure.[ii] ^{1}H NMR (500 MHz, Chloroform-d) δ 7.57 (d, J = 8.0 Hz, 1H), 7.29 (t, J = 7.9 Hz, 1H), 7.18 (d, J = 7.9 Hz, 1H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 132.90, 131.24, 130.29, 129.54, 125.13, 123.04, 120.77 (q, *J* = 310 Hz), 27.99 (q, *J* = 40.8 Hz). ^{19}F NMR (471 MHz, Chloroform-d) δ -63.41 – -67.46 (m).

V. Suzuki-Miyaura Coupling Conditions Screen



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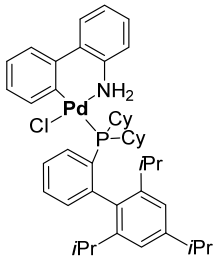
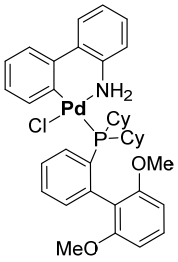
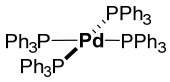


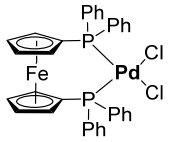
XPhos Pd G2



SPhos Pd G2



$Pd(PPh_3)_4$



$Pd(dppf)Cl_2$

In a nitrogen filled glovebox 10μL of 0.05 M stock solutions (0.5 μmol, 5 mol%) of four different Pd precatalysts ($Pd(PPh_3)_4$, XPhos Pd G2 and SPhos Pd G2 in THF; $Pd(dppf)Cl_2$ in CH_2Cl_2) were added to 1 mL glass inserts equipped with magnetic stir bars in a 24-well aluminum block. Following this, stock solutions of 8-chloro- or 8-bromo-quinoline (36.6 μL, 15 μmol, 1.5 equiv, 0.41M in THF), and phenylboronic acid pinacol ester or pheny boronic acid (100 μL, 10 μmol, 1.0 equiv) were added to the appropriate vials, and all solvents were evaporated under reduced presssure. Compound **1** was added to the dried vials as a stock solution in the appropriate reaction solvent (50 μL, 10 μmol, 1 equiv, 0.2M in DME or THF) followed by 30 μL of additional reaction solvent and 20 μL of K_3PO_4 or Na_2CO_3 solution (1.0 M aqueous, 20 μmol, 2 equiv). The reaction vials were sealed and agitated on a tumble stirrer at the desired temperature (25, 40 or 80 °C) for 24 hrs. The rxn vials were then cooled to ambient temperature and diluted with 500μL of DMSO containing biphenyl (10 μmol) as an internal standard, stirring vigorously for 5 min. A 10 μL aliquot was removed from each vial and diluted with 700 μL of MeCN and quantitative UPLC-MS analysis was performed.

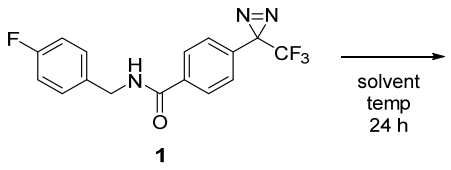
Results of Suzuki-Miyaura Coupling Screen

Entry	Temp (°C)	Ar-X, X =	Ph-B(OR)$_2$, (OR)$_2$ =	Catalyst	Base	Solvent	Ar-X LC A%[1]	**6** LC A%[1]	**7** LC A%[1]	**1** % remain
1	25	Br	$(OH)_2$	$Pd(dppf)Cl_2$	Na_2CO_3	DME	47	53	0	91
2	25	Br	$(OH)_2$	$Pd(PPh_3)_4$	Na_2CO_3	DME	54	46	0	86
3	25	Br	$(OH)_2$	SPhos Pd G2	K_3PO_4	THF	44	53	3	100
4	25	Br	$(OH)_2$	XPhos Pd G2	K_3PO_4	THF	31	62	7	87
5	25	Br	pin	$Pd(dppf)Cl_2$	Na_2CO_3	DME	45	55	0	103
6	25	Br	pin	$Pd(PPh_3)_4$	Na_2CO_3	DME	56	44	0	102
7	25	Br	pin	SPhos Pd G2	K_3PO_4	THF	48	49	3	102
8	25	Br	pin	XPhos Pd G2	K_3PO_4	THF	36	56	8	91
9	25	Cl	$(OH)_2$	$Pd(dppf)Cl_2$	Na_2CO_3	DME	97	3	0	39
10	25	Cl	$(OH)_2$	$Pd(PPh_3)_4$	Na_2CO_3	DME	100	0	0	55
11	25	Cl	$(OH)_2$	SPhos Pd G2	K_3PO_4	THF	82	0	18	75
12	25	Cl	$(OH)_2$	XPhos Pd G2	K_3PO_4	THF	50	4	47	39
13	25	Cl	pin	$Pd(dppf)Cl_2$	Na_2CO_3	DME	96	4	0	73
14	25	Cl	pin	$Pd(PPh_3)_4$	Na_2CO_3	DME	97	3	0	70
15	25	Cl	pin	SPhos Pd G2	K_3PO_4	THF	51	2	47	15
16	25	Cl	pin	XPhos Pd G2	K_3PO_4	THF	47	12	42	25
17	40	Br	$(OH)_2$	$Pd(dppf)Cl_2$	Na_2CO_3	DME	58	42	0	87*
18	40	Br	$(OH)_2$	$Pd(PPh_3)_4$	Na_2CO_3	DME	74	26	0	15*
19	40	Br	$(OH)_2$	SPhos Pd G2	K_3PO_4	THF	23	72	5	92
20	40	Br	$(OH)_2$	XPhos Pd G2	K_3PO_4	THF	22	70	9	83
21	40	Br	pin	$Pd(dppf)Cl_2$	Na_2CO_3	DME	50	50	0	97*
22	40	Br	pin	$Pd(PPh_3)_4$	Na_2CO_3	DME	50	50	0	50*
23	40	Br	pin	SPhos Pd G2	K_3PO_4	THF	42	54	3	101
24	40	Br	pin	XPhos Pd G2	K_3PO_4	THF	34	59	7	78
25	40	Cl	$(OH)_2$	$Pd(dppf)Cl_2$	Na_2CO_3	DME	94	6	0	92
26	40	Cl	$(OH)_2$	$Pd(PPh_3)_4$	Na_2CO_3	DME	97	3	0	92
27	40	Cl	$(OH)_2$	SPhos Pd G2	K_3PO_4	THF	38	3	59	5
28	40	Cl	$(OH)_2$	XPhos Pd G2	K_3PO_4	THF	41	6	53	22
29	40	Cl	pin	$Pd(dppf)Cl_2$	Na_2CO_3	DME	90	10	0	83*
30	40	Cl	pin	$Pd(PPh_3)_4$	Na_2CO_3	DME	94	6	0	33*
31	40	Cl	pin	SPhos Pd G2	K_3PO_4	THF	47	5	48	4
32	40	Cl	pin	XPhos Pd G2	K_3PO_4	THF	45	15	40	12
33	85	Br	$(OH)_2$	$Pd(dppf)Cl_2$	Na_2CO_3	DME	17	83	0	43
34	85	Br	$(OH)_2$	$Pd(PPh_3)_4$	Na_2CO_3	DME	25	75	0	35
35	85	Br	$(OH)_2$	SPhos Pd G2	K_3PO_4	THF	19	75	6	35
36	85	Br	$(OH)_2$	XPhos Pd G2	K_3PO_4	THF	20	70	10	29
37	85	Br	pin	$Pd(dppf)Cl_2$	Na_2CO_3	DME	34	66	0	46
38	85	Br	pin	$Pd(PPh_3)_4$	Na_2CO_3	DME	38	62	0	41
39	85	Br	pin	SPhos Pd G2	K_3PO_4	THF	33	63	4	40

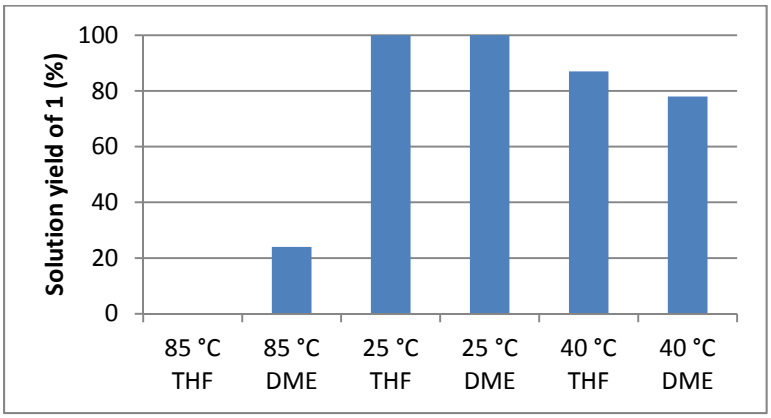
40	85	Br	pin	XPhos Pd G2	K3PO4	THF	33	60	7	36
41	85	Cl	$(OH)_2$	$Pd(dppf)Cl_2$	Na_2CO_3	DME	94	4	2	11
42	85	Cl	$(OH)_2$	$Pd(PPh_3)_4$	Na_2CO_3	DME	87	6	7	0
43	85	Cl	$(OH)_2$	SPhos Pd G2	K_3PO_4	THF	35	9	56	0
44	85	Cl	$(OH)_2$	XPhos Pd G2	K_3PO_4	THF	28	23	49	0
45	85	Cl	pin	$Pd(dppf)Cl_2$	Na_2CO_3	DME	93	3	3	20
46	85	Cl	pin	$Pd(PPh_3)_4$	Na_2CO_3	DME	89	4	7	14
47	85	Cl	pin	SPhos Pd G2	K_3PO_4	THF	44	7	49	0
48	85	Cl	pin	XPhos Pd G2	K_3PO_4	THF	40	16	43	3

[1]LC A% (liquid chromatography area percent) represents the percentage of integrtaed peak area (absorbance at 215 nM) observed in UPLC analyses for reaction mixtures, corresponding to each species (Ar-X, **6**, or **7**) relative to the total integrated peak area for all three. For example, LC A% Ar-X = LC A(Ar-X) / (LC A(Ar-X) + LC A(**6**) + LC A(**7**)) * 100.
*Two equivalents of **1** were added

VI. Thermal Stability of Diazirine 1



1

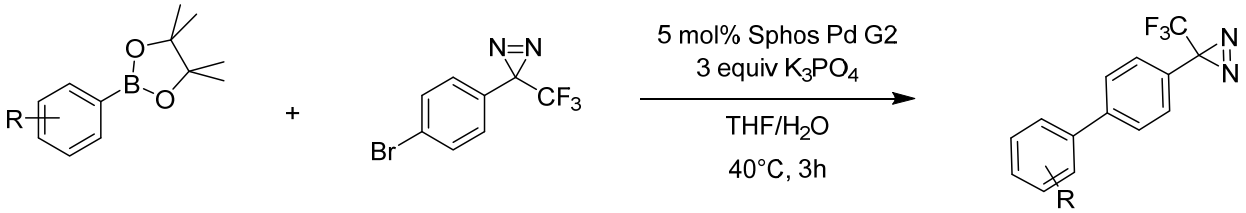


Entry	Solvent	Temp (ºC)	**1** recovered (%)
1	THF	85	0
2	DME		24
3	THF	25	100
4	DME		100
5	THF	40	87
6	DME		78

In a nitrogen filled glovebox, stock solutions of Compound **1** (50 µL, 10 µmol, 1 equiv, 0.2M in DME or THF) were added to 1 mL glass inserts equipped with magnetic stir bars in a 24-well aluminum block. The reaction vials were sealed and agitated on a tumble stirrer at the desired temperature (25, 40 or 80 °C) for 24 hrs. The rxn vials were then cooled to ambient temperature and diluted with 500µL of DMSO containing biphenyl (10 µmol) as an internal standard, stirring vigorously for 5 min. A 10 µL

aliquot was removed from each vial and diluted with 700 µL of MeCN and quantitative UPLC-MS analysis was performed.

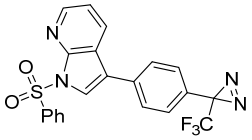
VII. Suzuki-Miyaura Cross-Coupling Screen with ArBpin Chemistry Informer Library



In a nitrogen filled glovebox to an aluminum block holding 1 mL reaction vial inserts containing aryl pinacol boronates (10 µmol) and magnetic stir bars, was added 36.6 µL of a solution of diazirine **8** (0.41 M in THF, 15 µmol, 1.5 equiv.) followed by 10 µL of palladium SPhos G2 precatalyst solution (0.05 M in THF, 0.50 µmol, 5 mol%), followed by 30 µL of K_3PO_4 (1.0 M aqueous, 30 µmol, 3 equiv). The reaction vials were sealed and stirred 40 °C for 3 hrs. The rxn vials were then cooled to r.t. and diluted with 500µL of DMSO-d6 containing 1,1,1-trifluorotoluene (0.02 M; 10 µmol) internal standard and analyzed by quantitative ^{19}F NMR spectroscopy to determine the solution yield. Results are given in Scheme 2.

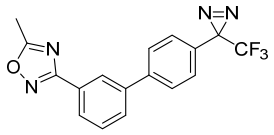
VIII. General Procedure for Preparative Suzuki-Miyaura Cross-Coupling of Diazirine 8 with Selected Members of ArBpin Chemistry Informer Library. To an 8 mL vial equipped with a stir bar, Ar-Bpin (0.1-0.4 mmol), SphosPd G2 (0.05 mol%) and solid K_3PO_4 (3 equiv) were added. The mixture was then brought into a glovebox and was dissolved in degassed THF and distilled water (0.1M, 3:1). Diazirine **8** was added (1.5 equiv) and the reaction was heated at 40 °C for 3 hours. After the reaction was cooled, EtOAc (5 mL) was added and washed with 10mL of H_2O. The aqueous layer was extracted 3 times with EtOAc and then the combined organic layers were washed with brine and dried over $MgSO_4$. The solvent was evaporated in vacuo and the crude mixture was purified using flash column chromatography (SiO_2 gel, 30% isocratic EtOAc/hexane for 10 minutes, then 50-100% EtOAc/hexane gradient over 7 minutes). After this treatment, compounds **3**, **6**, **7** contained ~5% impurities and were re-purified using reverse phase HPLC using 50% H_2O/CH_3CN over 16 minutes.

IX. Compound Characterization

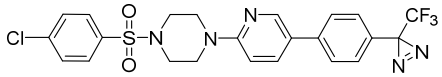


1-(phenylsulfonyl)-3-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)phenyl)-1H-pyrrolo[2,3-b]pyridine (10): 0.182 mmol scale, 46.3mg (57%) isolated as bright yellow crystalline solid. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.52 (d, *J* = 3.9 Hz, 1H), 8.28 (d, *J* = 7.8 Hz, 2H), 8.12 – 8.06 (m, 1H), 7.94 (s, 1H), 7.65 (d, *J* = 8.3 Hz, 2H), 7.61 (d, *J* = 7.2 Hz, 1H), 7.53 (t, *J* = 7.8 Hz, 2H), 7.32 (d, *J* = 8.2 Hz, 2H), 7.30 – 7.26 (m, 1H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 147.57, 145.49, 138.16, 134.22 (d, *J* = 1.6 Hz), 129.10, 128.66, 128.41, 128.17, 127.72, 127.30 – 127.10 (m), 123.18, 121.14, 119.33, 28.41 (q, *J* =

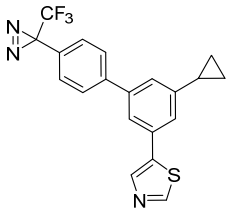
40.5 Hz). ^{19}F NMR (471 MHz, Chloroform-*d*) δ -65.10. HRMS (ESI) $[M+H]^+$ calculated for $C_{21}H_{14}F_3N_4O_2S^+$ 443.0784, found 443.0806.



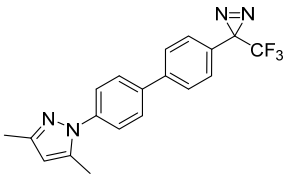
5-methyl-3-(4'-(3-(trifluoromethyl)-3H-diazirin-3-yl)-[1,1'-biphenyl]-3-yl)-1,2,4-oxadiazole (17): 0.276 mmol scale, 48.3 mg (50%) isolated as a white powder. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.30 (s, 1H), 8.10 (d, *J* = 7.6 Hz, 1H), 7.71 (t, *J* = 7.9 Hz, 3H), 7.59 (t, *J* = 7.7 Hz, 1H), 7.41 – 7.21 (m, 3H), 2.70 (s, 3H). ^{19}F NMR (471 MHz, Chloroform-*d*) δ -65.12. ^{13}C NMR (126 MHz, Chloroform-d) δ 176.72, 168.19, 141.55, 140.45, 129.73 (2C), 129.55, 128.52, 127.57, 126.96, 126.82, 126.02, 124.31 (q, *J* = 295 Hz), 28.41 (q, *J* = 40.5 Hz), 12.45. HRMS (ESI) $[M+H]^+$ calculated for $C_{17}H_{12}F_3N_4O^+$ 345.0958, found 345.0956.



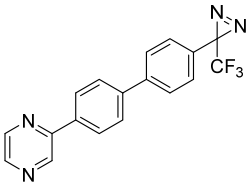
1-((4-chlorophenyl)sulfonyl)-4-(5-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)phenyl)pyridin-2-yl)piperazine (19): 0.216 mmol scale, 45mg (40%) isolated as a beige solid. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.40 (s, 1H), 7.74 (d, *J* = 7.8 Hz, 2H), 7.70 (d, *J* = 8.7 Hz, 1H), 7.54 (d, *J* = 8.4 Hz, 4H), 7.25 (d, *J* = 8.0 Hz, 2H), 6.69 (d, *J* = 8.7 Hz, 1H), 3.74 (s, 4H), 3.16 (s, 4H). ^{13}C NMR (126 MHz, Chloroform-d) δ 158.01, 146.28, 139.71, 139.36, 136.21, 134.04, 129.51, 129.17, 127.64, 127.07, 126.42, 125.47, 122.12 (q, *J* = 365 Hz), 106.97, 45.73, 44.66, 28.38 (q, *J* = 40.4 Hz). ^{19}F NMR (471 MHz, Chloroform-*d*) δ -65.20. HRMS (ESI) $[M+H]^+$ calculated for $C_{23}H_{20}ClF_3N_5O_2S^+$ 522.0973, found 522.0948.



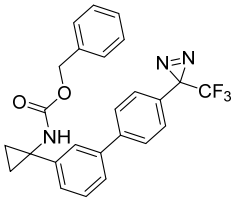
5-(5-cyclopropyl-4'-(3-((difluoro-l3-methyl)-l2-fluoranyl)-3H-diazirin-3-yl)-[1,1'-biphenyl]-3-yl)thiazole (22): 0.306 mmol scale, 82 mg (66%) isolated as brown oil. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.80 (s, 1H), 8.13 (s, 1H), 7.64 (d, *J* = 8.3 Hz, 2H), 7.53 (s, 1H), 7.31 (d, *J* = 7.5 Hz, 3H), 7.24 (s, 1H), 2.03 (td, *J* = 8.5, 4.3 Hz, 1H), 1.09 (q, *J* = 6.2 Hz, 2H), 0.83 (q, *J* = 5.1 Hz, 2H). ^{13}C (176 Hz, $CDCl_3$) δ 153.7, 147.2, 142.9, 141.4, 140.7, 139.9, 132.9, 128.8, 127.9, 127.8, 124.7, 125.3, 124.3, 123.7, 29.3, 16.0, 10.3. ^{19}F NMR (470 MHz, Chloroform-*d*) δ -65.15.HRMS (ESI) $[M+H]^+$ calculated for $C_{20}H_{15}F_3N_3S^+$ 386.0933, found 386.0914.



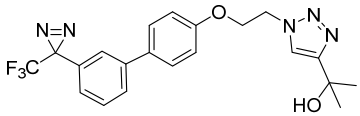
3,5-dimethyl-1-(4'-(3-(trifluoromethyl)-3H-diazirin-3-yl)-[1,1'-biphenyl]-4-yl)-1H-pyrazole (24): 0.325 mmol scale, 78.7 mg (67%) isolated as pale yellow crystalline solid. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.66 (d, *J* = 8.4 Hz, 4H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.31 (d, *J* = 8.2 Hz, 2H), 6.05 (s, 1H), 2.38 (s, 3H), 2.34 (s, 3H). ^{13}C NMR (126 MHz, Chloroform-d) δ 149.31, 141.55, 139.77, 139.40, 138.41, 128.29, 127.66, 127.42, 126.71, 124.91, 122.10 (q, *J* = 302 Hz), 107.35, 28.41 (q, *J* = 40.5 Hz), 13.55, 12.55. ^{19}F NMR (470 MHz, Chloroform-*d*) δ -65.15. HRMS (ESI) $[M+H]^+$ calculated for $C_{19}H_{16}F_3N_4^+$ 357.1322, found 357.1320.



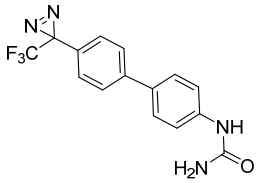
2-(4'-(3-(trifluoromethyl)-3H-diazirin-3-yl)-[1,1'-biphenyl]-4-yl)pyrazine (27): 0.354 mmol scale, 84mg (70%) isolated as white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.75 (s, 1H), 8.65 (s, 1H), 8.28 (d, *J* = 7.6 Hz, 2H), 7.90 (dd, *J* = 15.0, 7.9 Hz, 4H), 7.40 (d, *J* = 7.6 Hz, 2H). ^{19}F NMR (471 MHz, DMSO-d_6) δ -64.49. ^{13}C NMR (126 MHz, DMSO-d_6) δ 150.8, 144.38, 143.60, 142.12, 141.02, 139.88, 135.61, 127.63, 127.47, 127.38, 127.06, 127.01, 121.91 (q, *J* = 274.8 Hz), 28.08 (q, *J* = 40.0 Hz). HRMS (ESI) $[M+H]^+$ calculated for $C_{18}H_{12}F_3N_4^+$ 341.1009, found 341.1017.



benzyl (1-(4'-(3-(trifluoromethyl)-3H-diazirin-3-yl)-[1,1'-biphenyl]-3-yl)cyclopropyl)carbamate (31): 0.153 mmol scale, 37.6mg (54%) isolated as yellow solid. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.57 (d, *J* = 7.4 Hz, 2H), 7.46 (s, 1H), 7.43 – 7.14 (m, 10H), 5.13 (s, 2H), 1.48 – 1.16 (m, 4H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 156.02, 143.56, 142.63, 139.97, 136.40, 129.07, 128.56, 128.28, 128.22, 127.99, 127.59, 126.84, 125.33, 124.95, 124.43, 122.18 (q, *J* = 274.7 Hz), 66.75, 35.56, 29.72, 28.42 (q, *J* = 40.5 Hz), 18.21. ^{19}F NMR (471 MHz, Chloroform-*d*) δ -65.13. HRMS (ESI) $[M+H]^+$ calculated for $C_{25}H_{21}F_3N_3O_2^+$ 452.1580, found 452.1588.



2-(1-(2-((3'-(3-(trifluoromethyl)-3H-diazirin-3-yl)-[1,1'-biphenyl]-4-yl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)propan-2-ol (34): 0.268 mmol scale, 44.1 mg (38%) isolated yield as an yellow oil, found to be an inseparable mixture of **34** and boronate ester starting material. Spectral data reported for product **34**. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.77 (d, *J* = 7.9 Hz, 1H), 7.65 (d, *J* = 14.5 Hz, 1H), 7.59 (d, *J* = 7.6 Hz, 1H), 7.53 - 7.44 (m, 2H), 7.31 (s, 1H), 7.20 (d, *J* = 7.7 Hz, 1H), 6.98 (d, *J* = 8.1 Hz, 1H), 6.89 (d, *J* = 8.0 Hz, 1H), 4.86 - 4.67 (m, 2H), 4.49 - 4.34 (m, 2H), 2.75 (m, 1H), 1.66 (d, *J* = 6.3 Hz, 6H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 157.96, 141.43, 133.58, 129.75, 129.73, 129.43, 128.52, 128.18, 125.03, 124.80, 122.24 (q, *J* = 274.7 Hz), 120.39, 115.10, 68.60, 66.63, 49.73, 30.57, 28.61 (q, *J* = 40.3 Hz). ^{19}F NMR (471 MHz, Chloroform-d) δ -65.11. HRMS (ESI) $[M+H]^+$ calculated for $C_{21}H_{21}F_3N_5O_2^+$ 432.1642, found 432.1633.



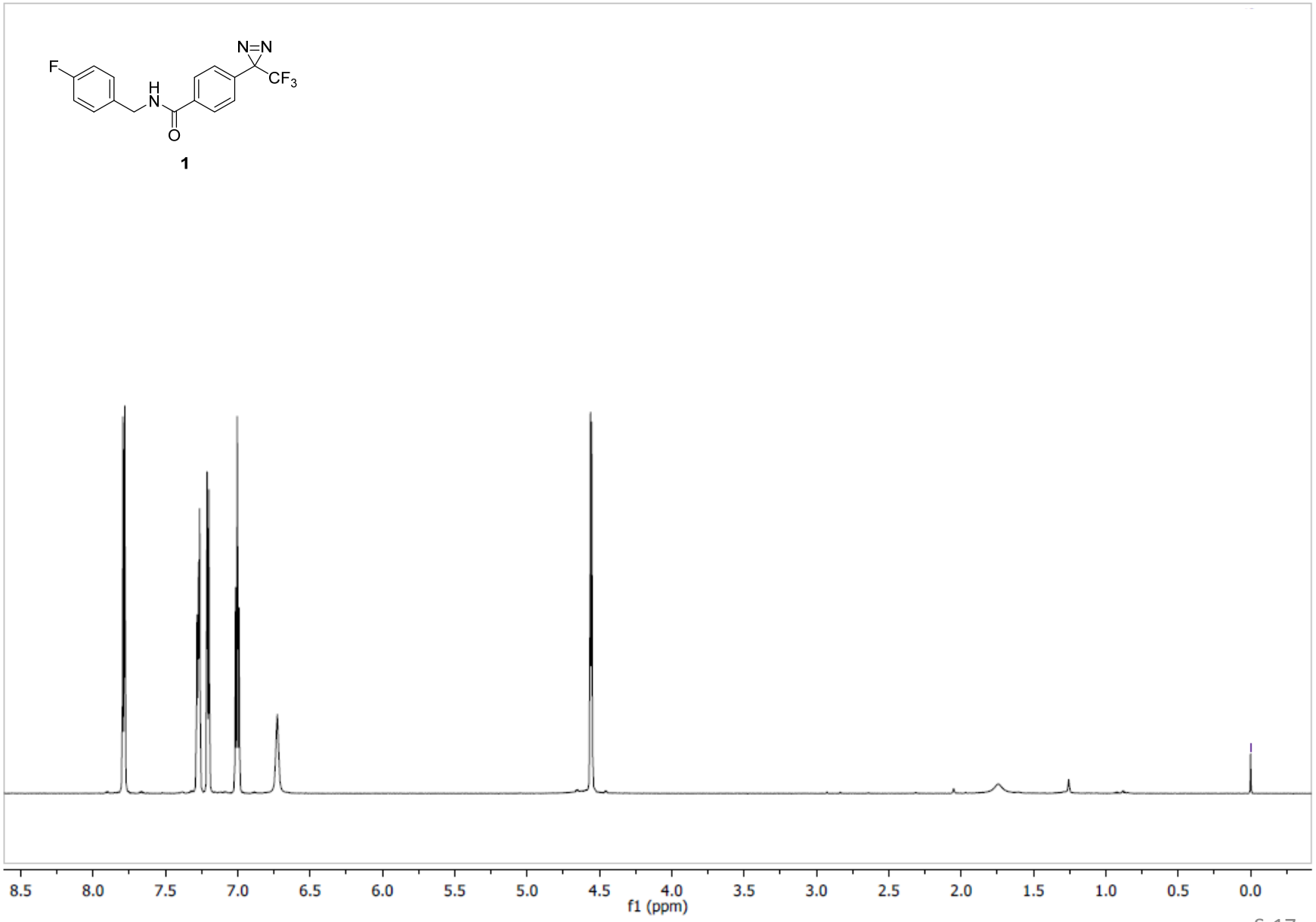
1-(4'-(3-(trifluoromethyl)-3H-diazirin-3-yl)-[1,1'-biphenyl]-4-yl)urea (36): 0.214 mmol scale, 30.8 mg (45%) isolated as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70 (s, 1H), 7.76 (d, *J* = 8.4 Hz, 2H), 7.59 (d, *J* = 8.6 Hz, 2H), 7.52 (d, *J* = 8.7 Hz, 2H), 7.32 (d, *J* = 8.2 Hz, 2H), 5.91 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.84, 141.76, 140.90, 130.98, 127.07, 126.89, 126.75, 125.60, 121.95 (q, *J* = 274.7 Hz), 118.02, 28.06 (q, *J* = 39.8 Hz). ^{19}F NMR (471 MHz, DMSO-d_6) δ -64.55. HRMS (ESI) $[M+H]^+$ calculated for C15H12F3N4O+ 321.0963, found 321.0958.

X. References

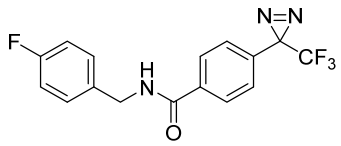
[1] Hill, J. R.; Robertson, A. A. B. *J. Med. Chem.* **Article ASAP.** DOI: 10.1021/acs.jmedchem.7b01561
[2] https://cdn-pubs.acs.org/doi/10.1021/ci00057a005
[3] http://www.daylight.com/dayhtml/doc/theory/theory.smarts.html
[4] Zhang, Y.; Gao, J.; Li, W.; Lee, H.; Lu, B. Z.; Senanayake, C. H. *J. Org. Chem*. **2011**, 76, 6394–6400.
[5] Bender, T.; Huss, M.; Wieczorek, H.; Grond, S.; von Zezschwitz, P. *Eur. J. Org. Chem.* **2007**, 3870-3878.

XI. Spectroscopic Data

^{1}H NMR (700 MHz, Chloroform-*d*)



^{13}C NMR (176 MHz, Chloroform-*d*)



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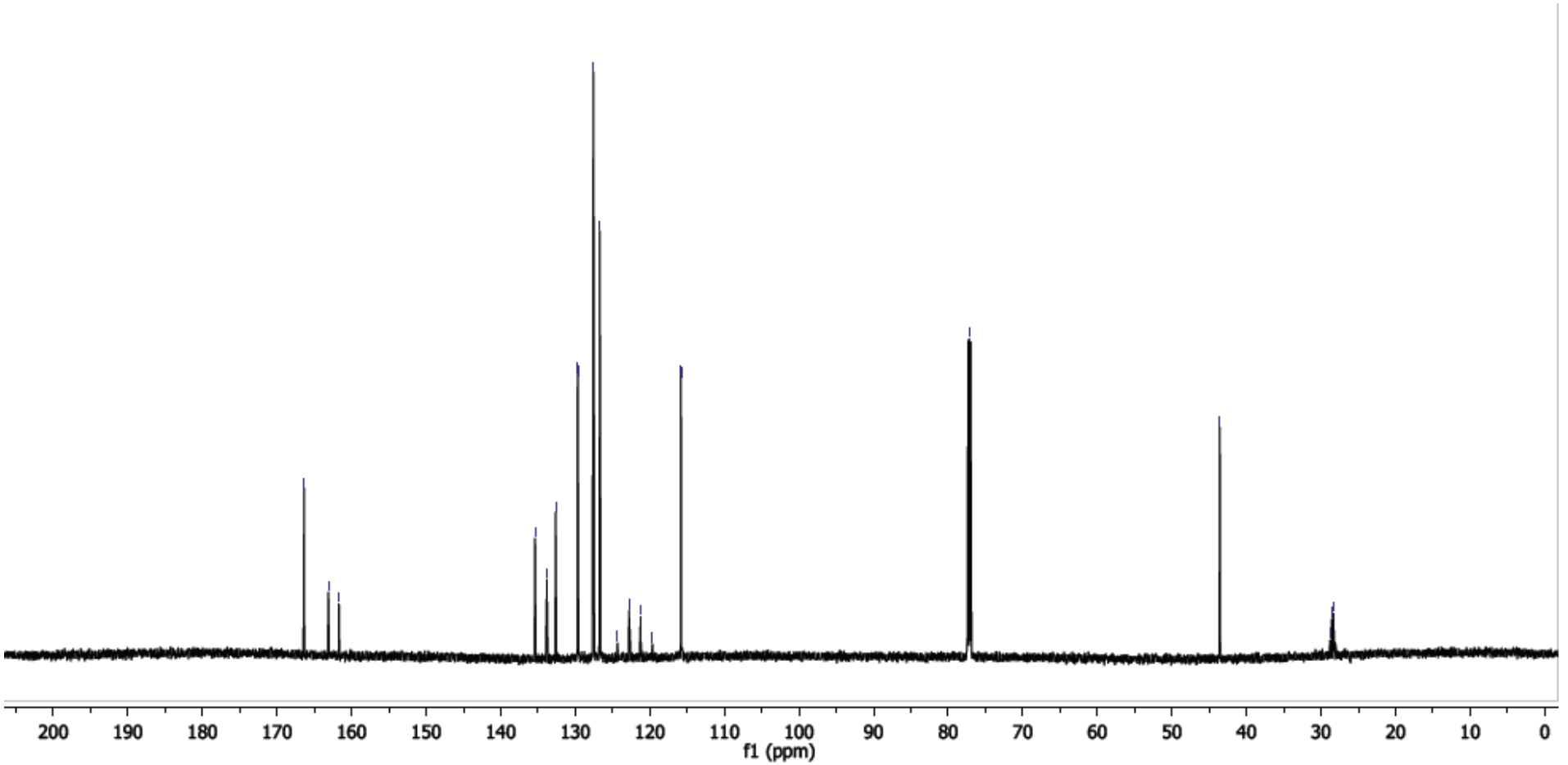
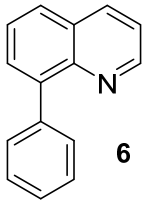


^{1}H NMR (500 MHz, Chloroform-*d*)



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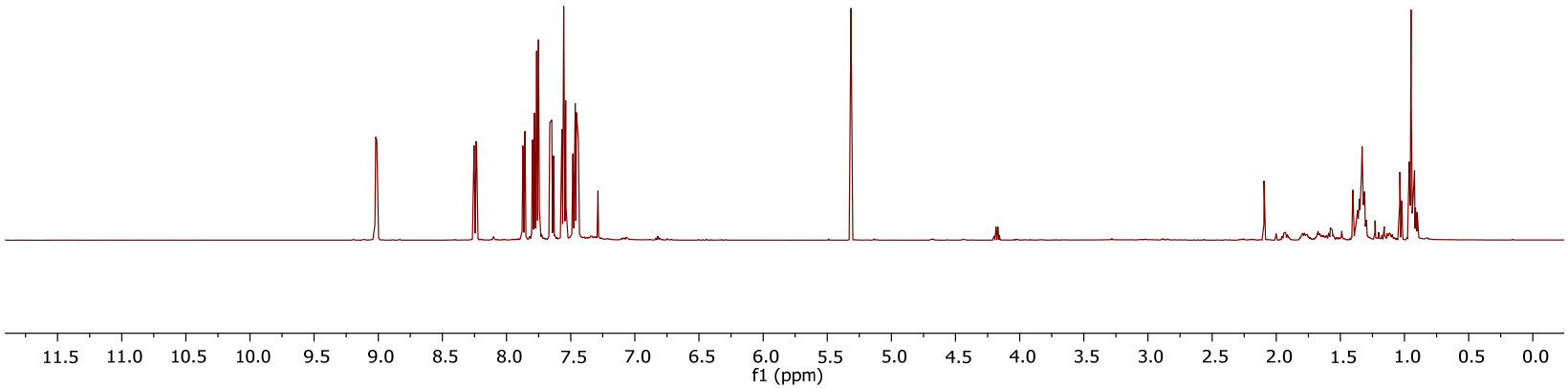
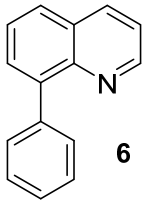


^{13}C NMR (126 MHz, Chloroform-*d*)



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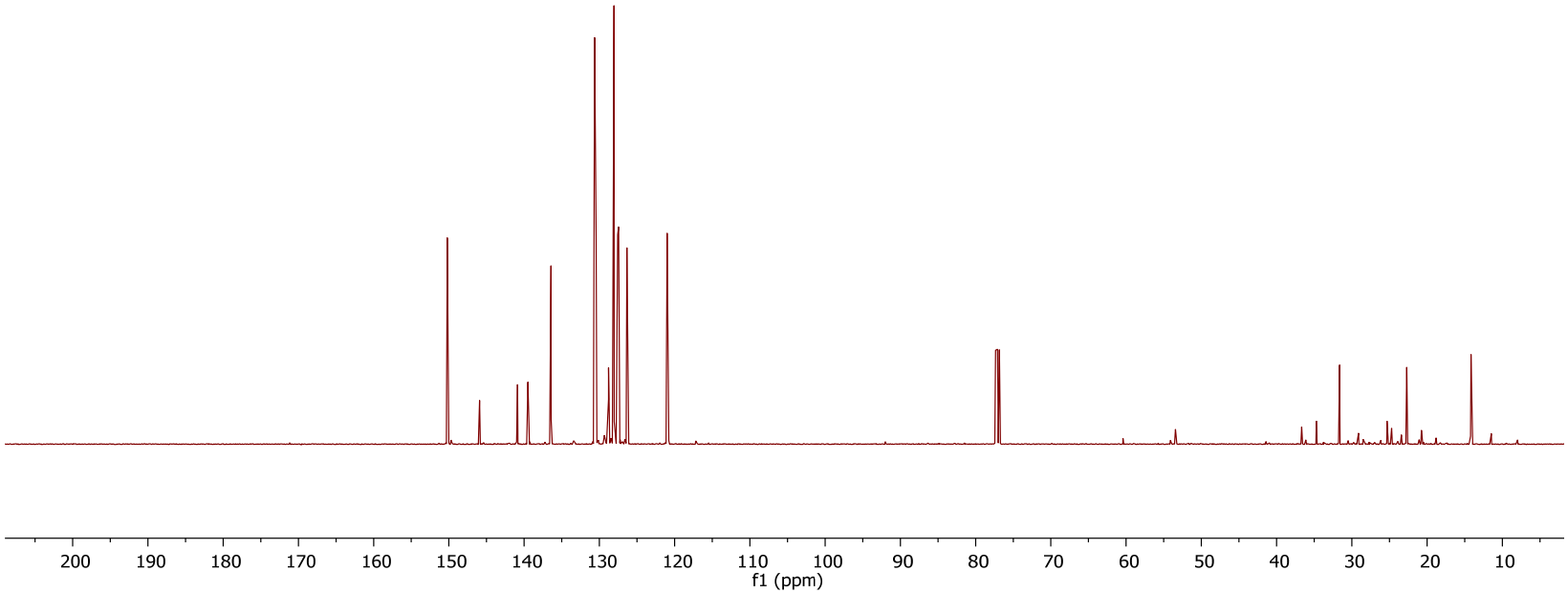
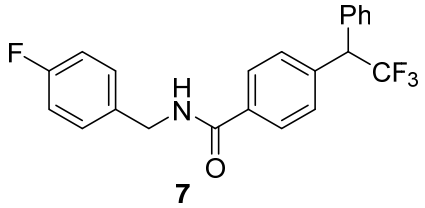


^{1}H NMR (500 MHz, Chloroform-*d*)



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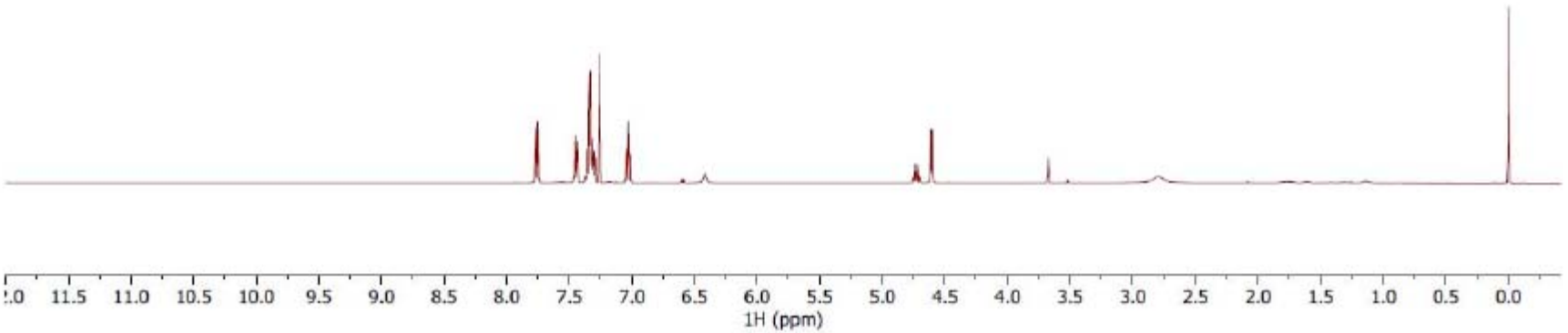
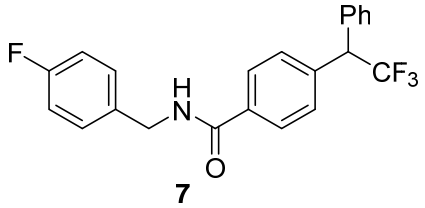


^{13}C NMR (126 MHz, Chloroform-*d*)



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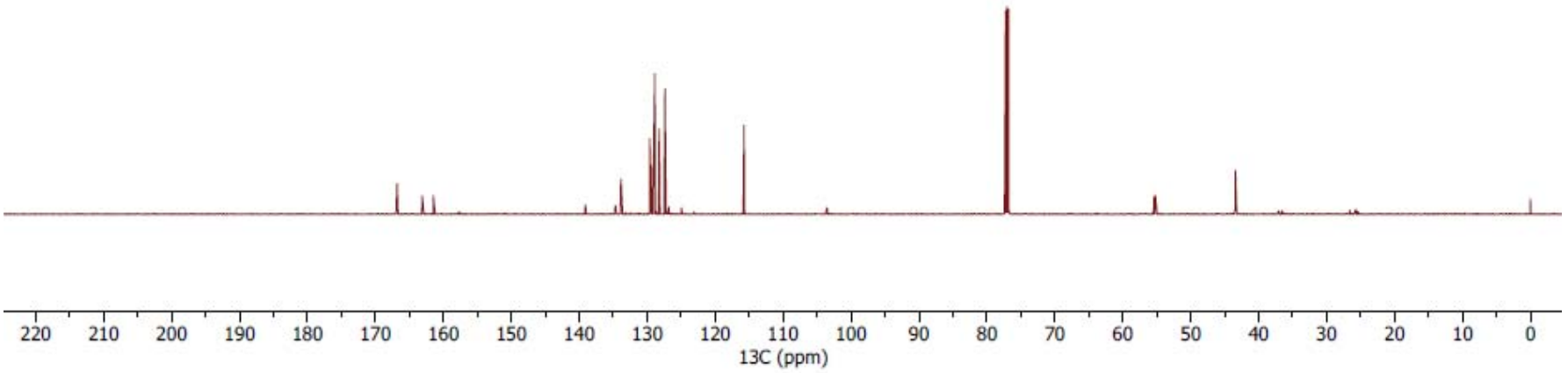
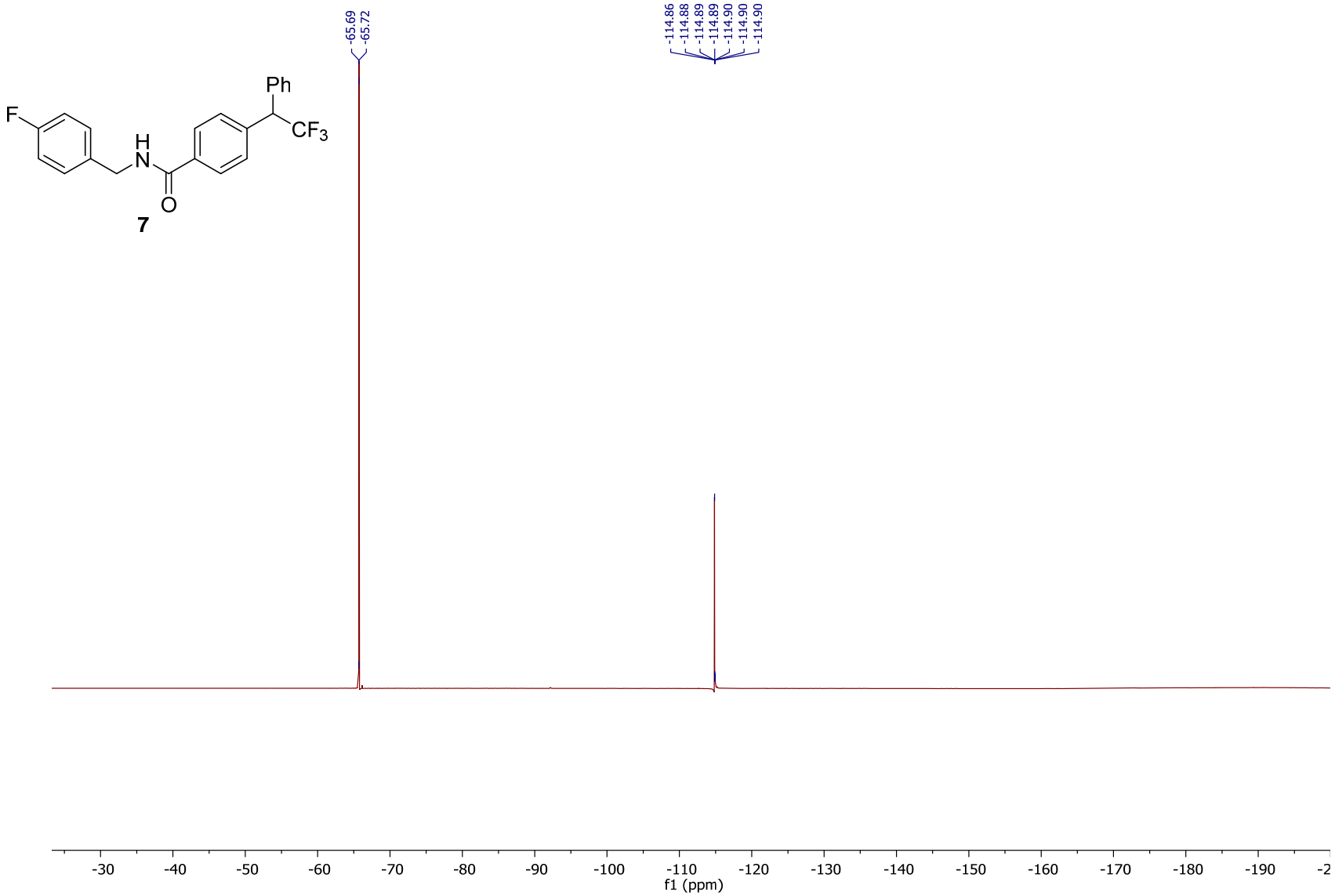
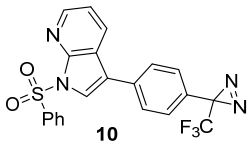


^{19}F NMR (471 MHz, Chloroform-*d*).



^{1}H NMR (500 MHz, Chloroform-*d*)



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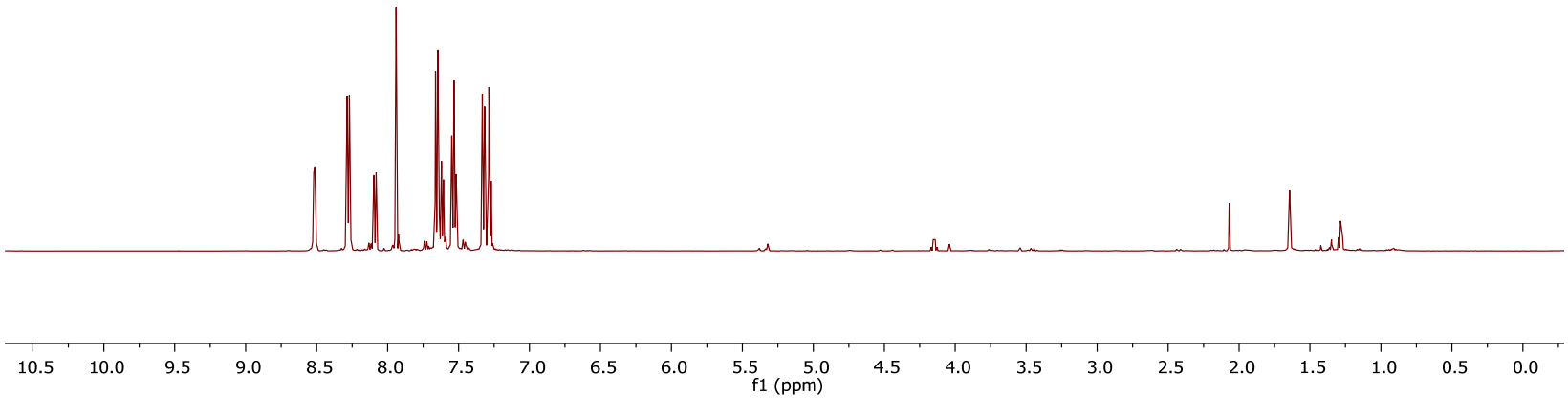
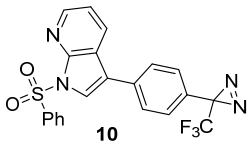


^{13}C NMR (126 MHz, Chloroform-*d*)



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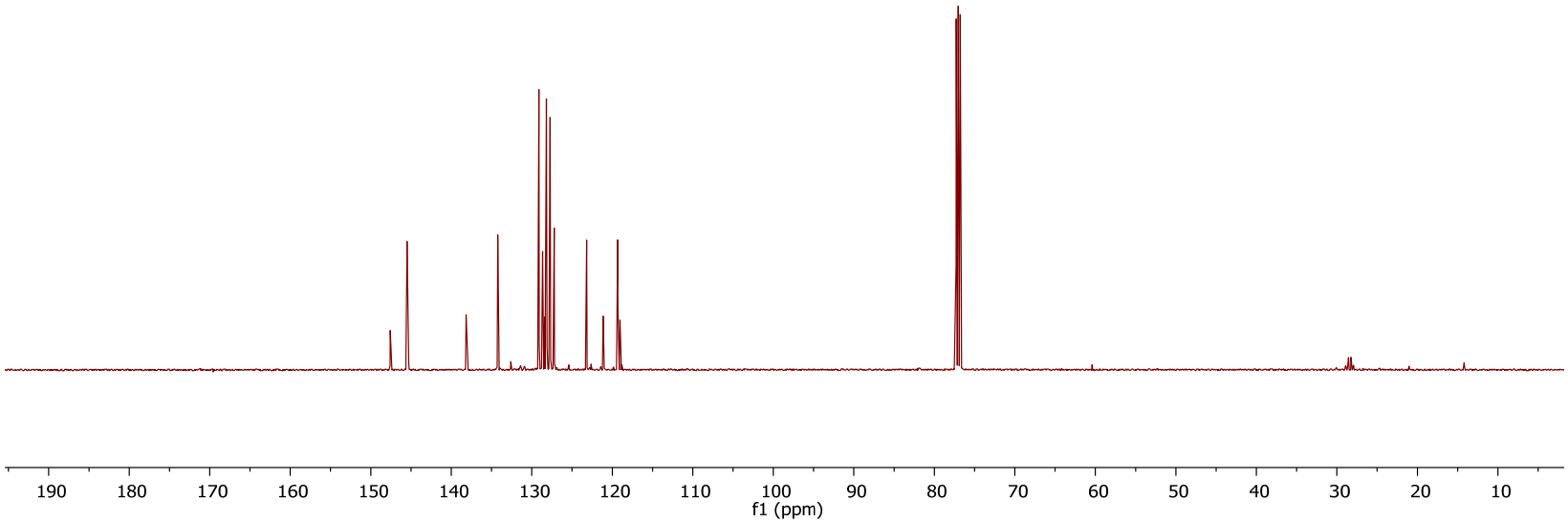
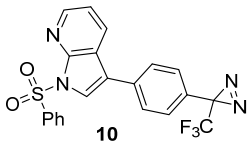


^{19}F NMR (470 MHz, Chloroform-*d*)



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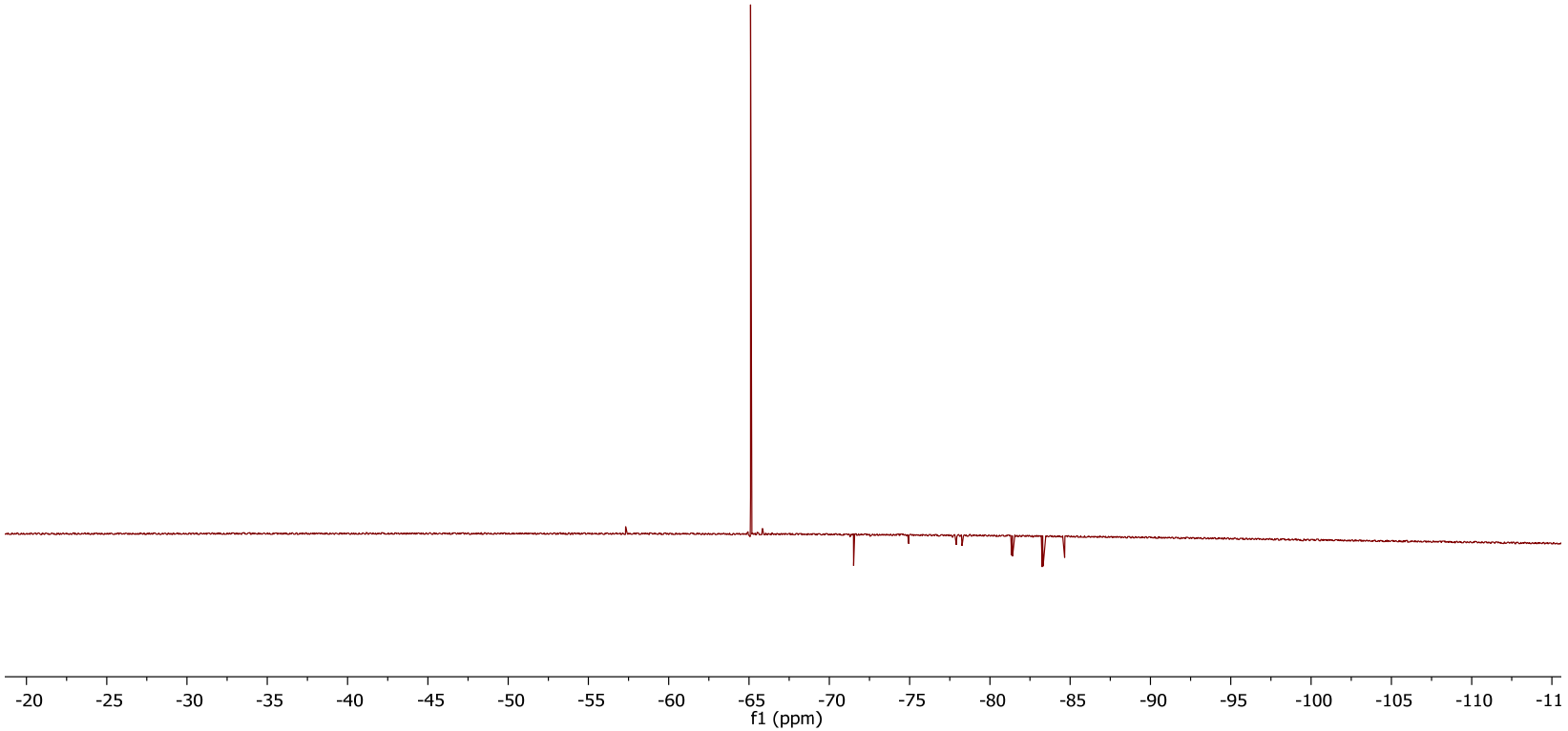
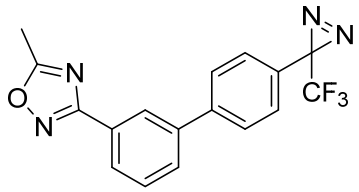


^{1}H NMR (500 MHz, Chloroform-*d*)



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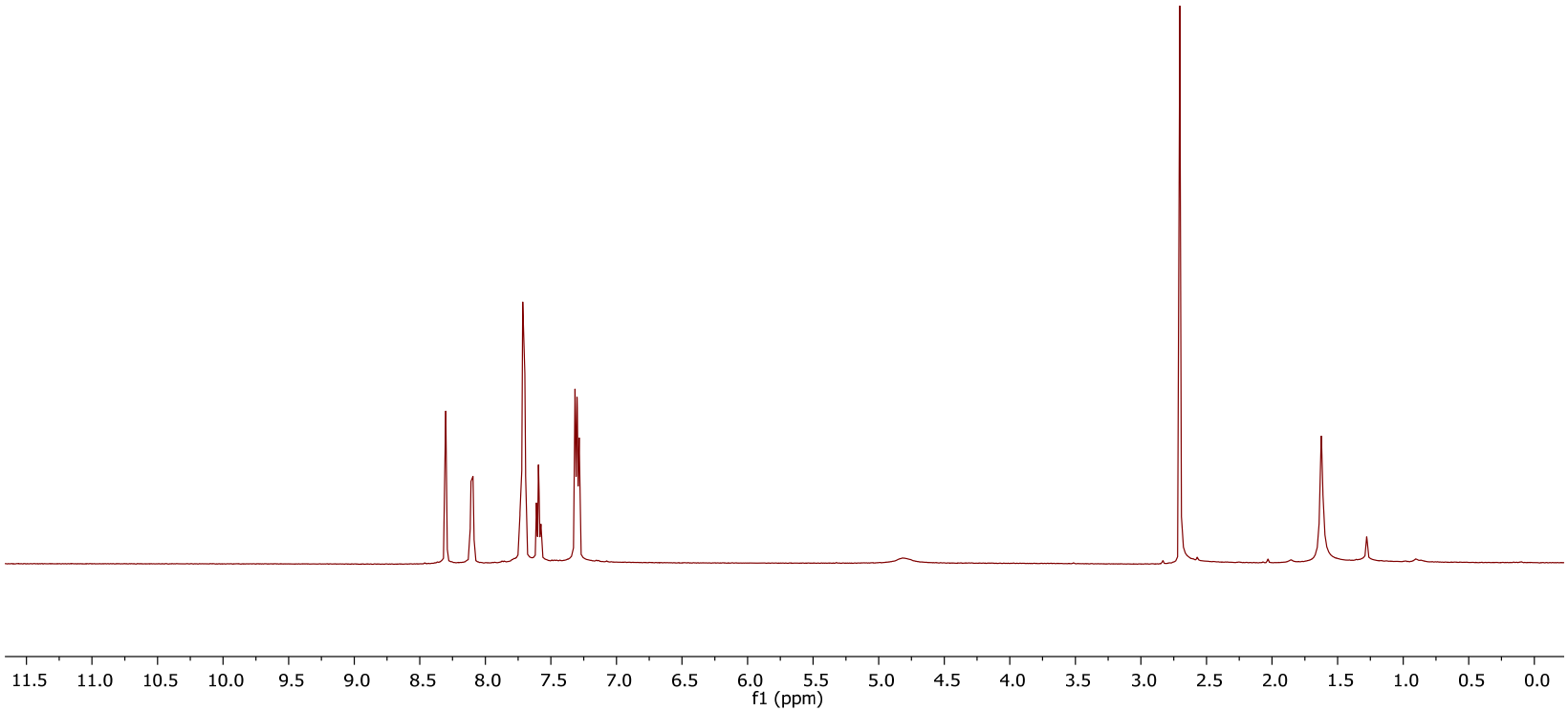
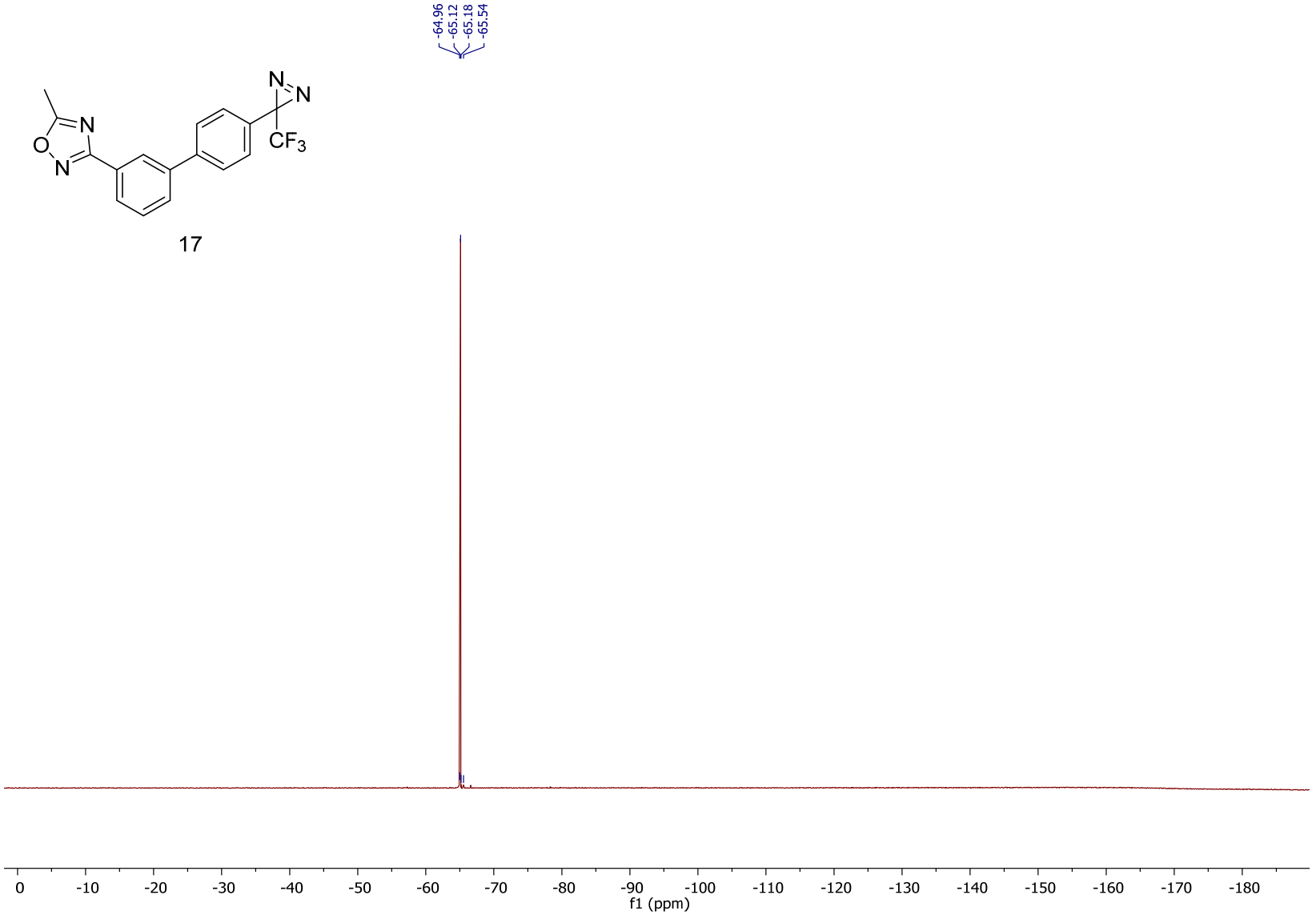
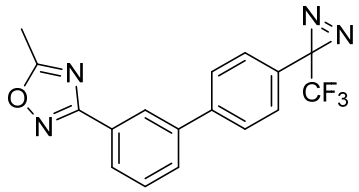


^{19}F NMR (470 MHz, Chloroform-*d*)



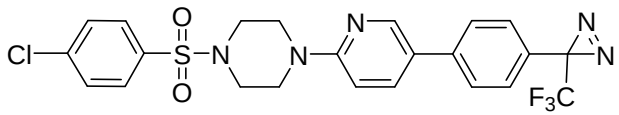
17

^{13}C NMR (126 MHz, Chloroform-*d*)



17

^{1}H NMR (500 MHz, Chloroform-*d*)



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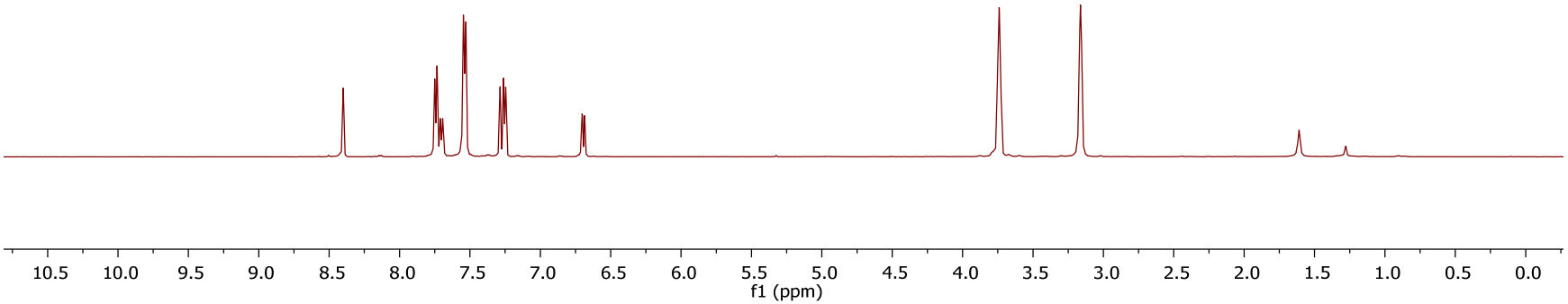
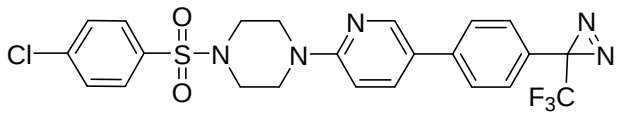
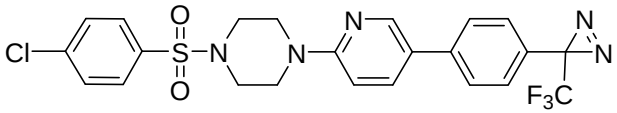


^{13}C NMR (126 MHz, Chloroform-*d*)



19

^{19}F NMR (470 MHz, Chloroform-*d*)



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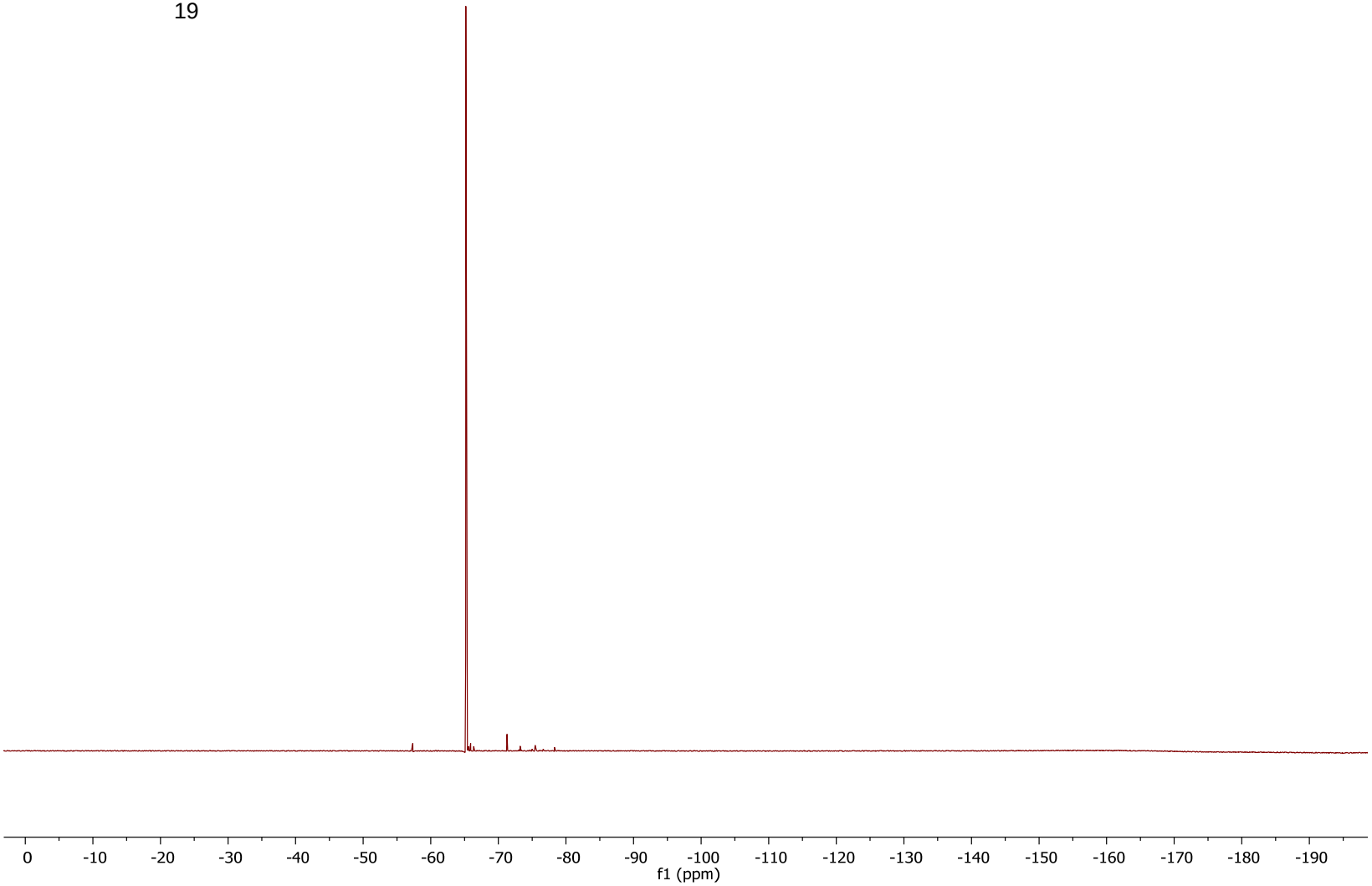
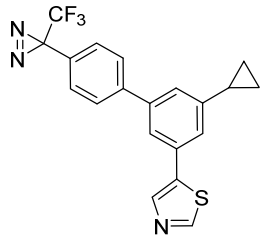


^{1}H NMR (500 MHz, Chloroform-*d*)



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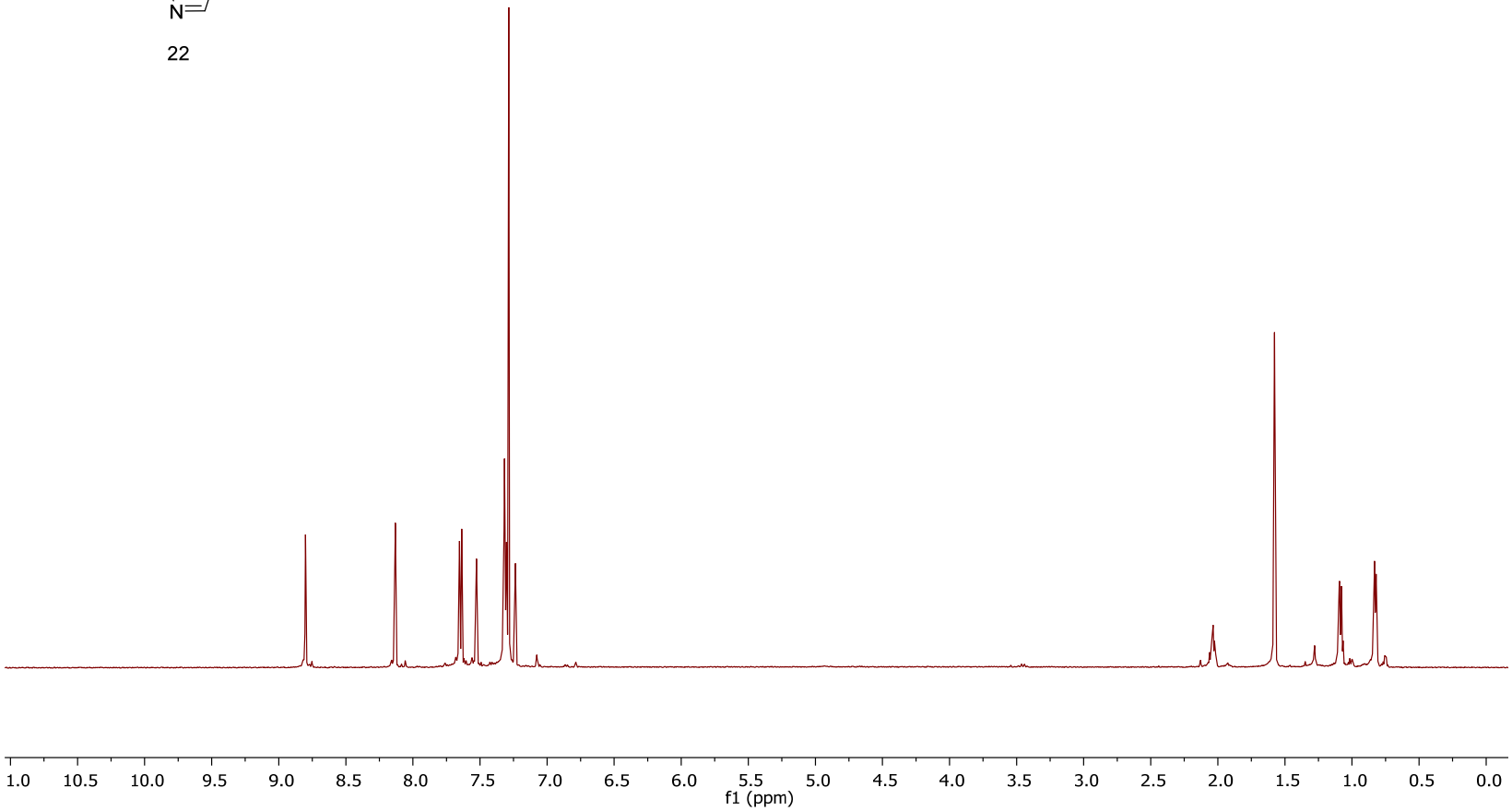
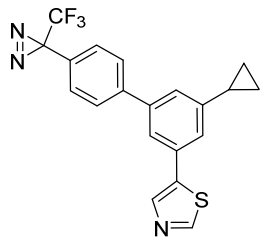


^{13}C NMR (126 MHz, Chloroform-*d*)



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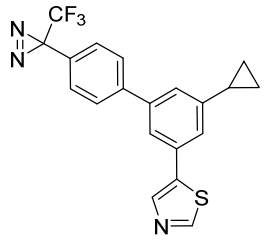
^{19}F NMR (470 MHz, Chloroform-*d*)



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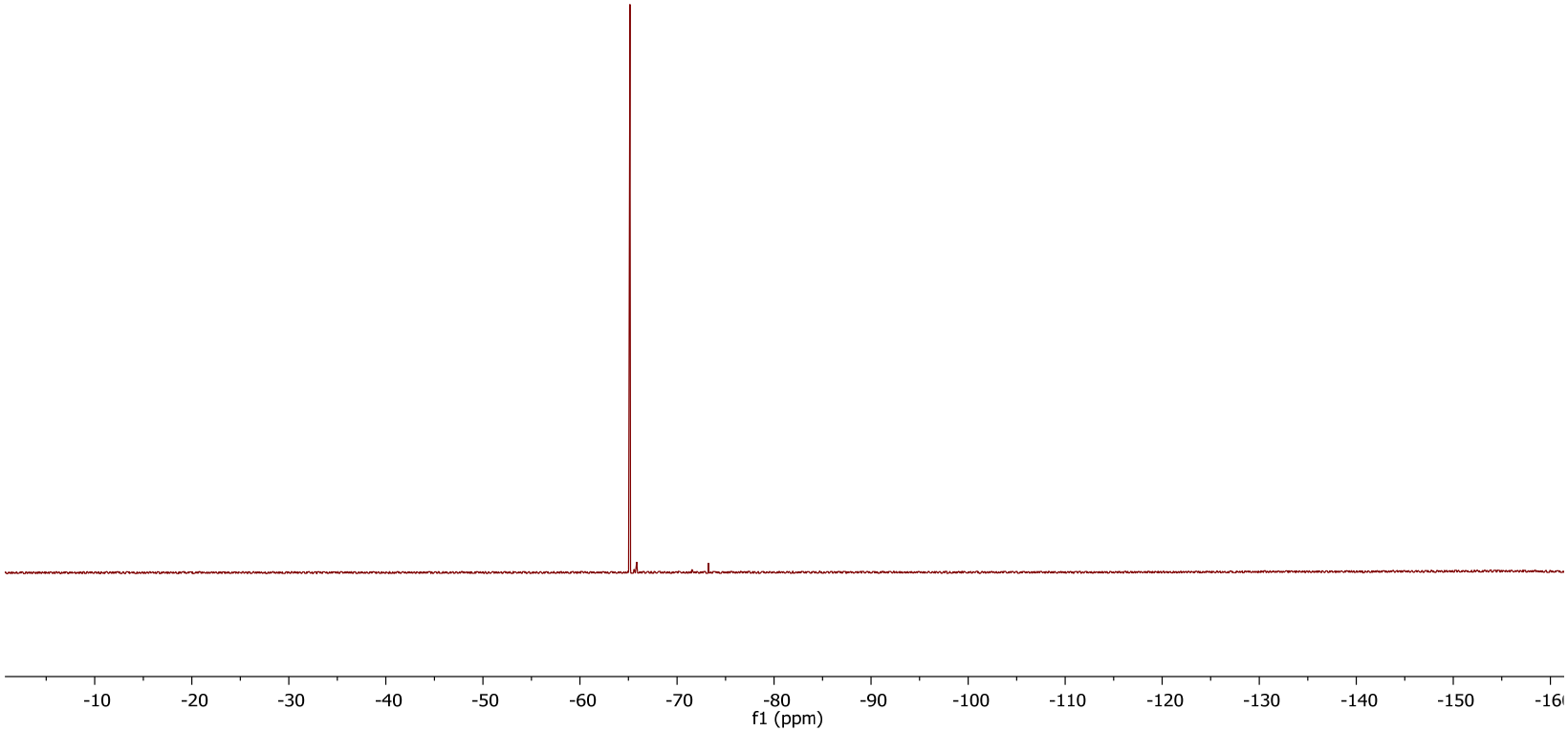
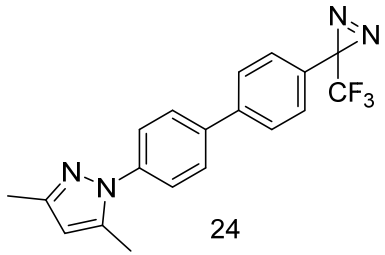


^{1}H NMR (500 MHz, Chloroform-*d*)



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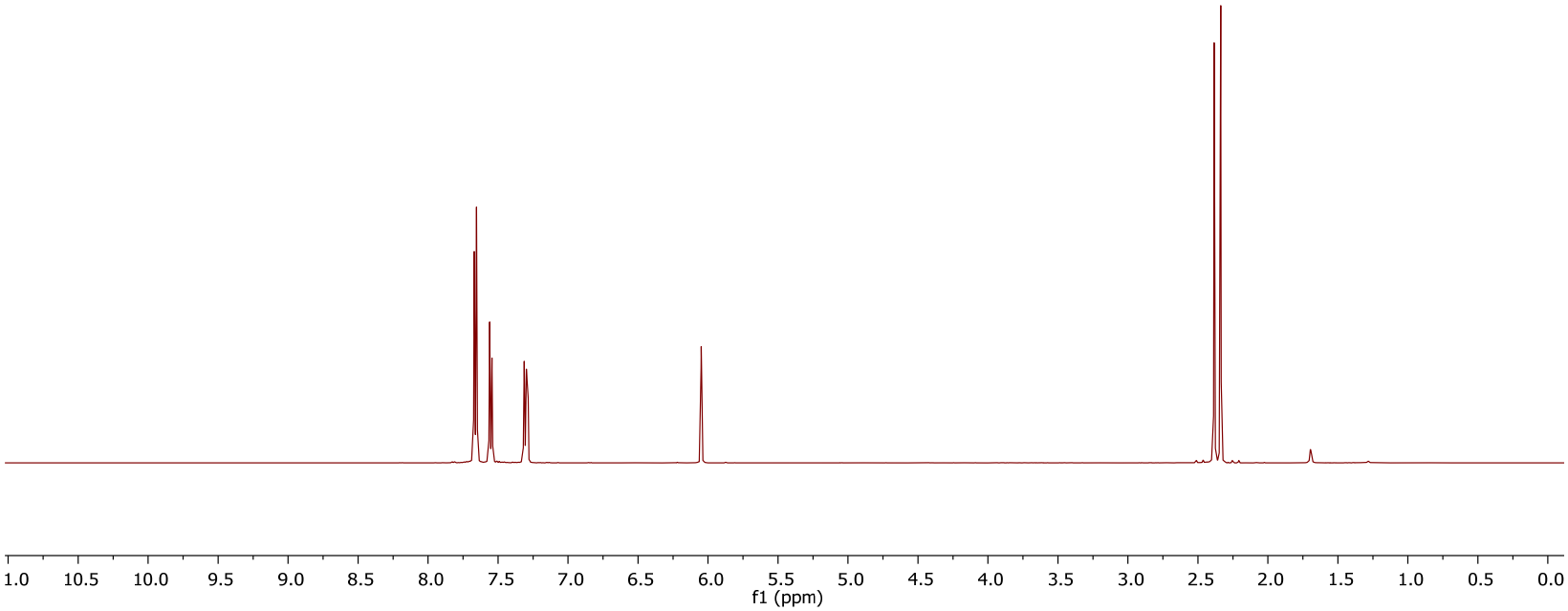
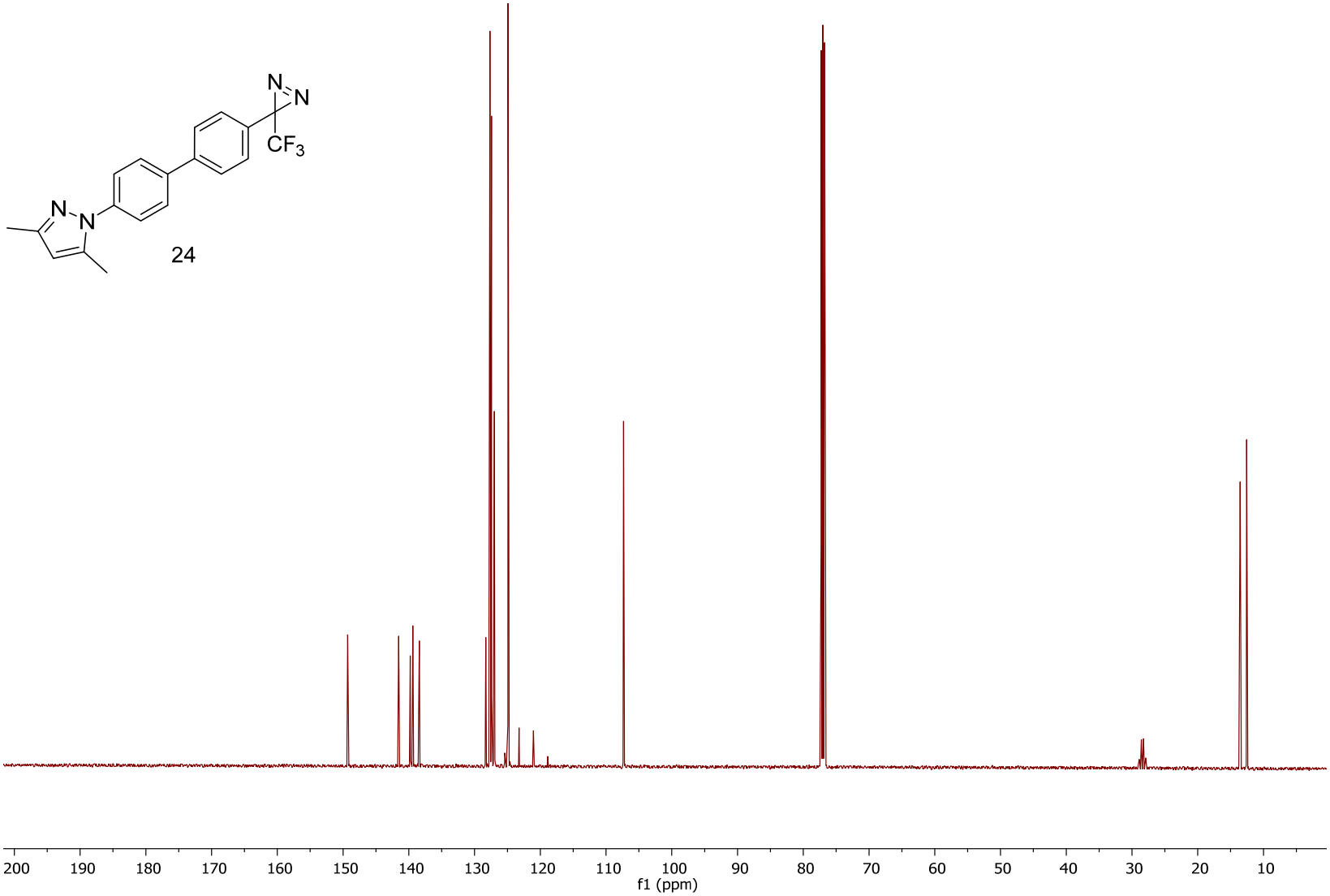
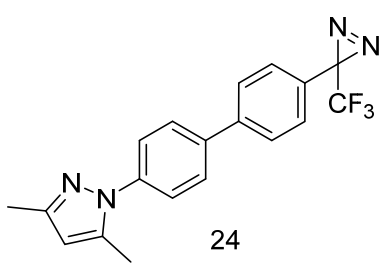


^{13}C NMR (126 MHz, Chloroform-*d*)

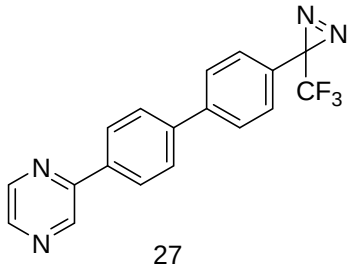


^{19}F NMR (470 MHz, Chloroform-*d*)



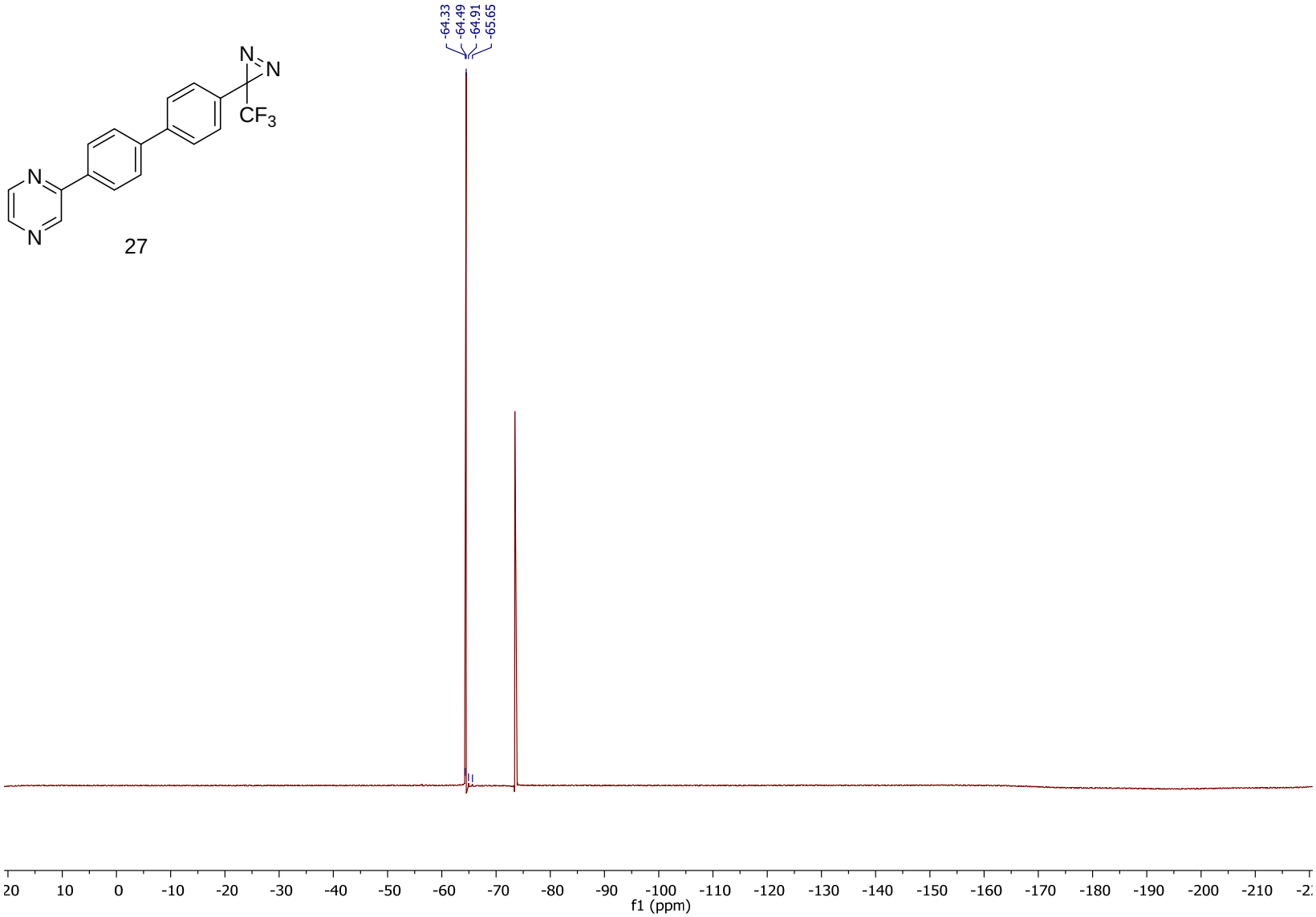
24

^{1}H NMR (500 MHz, Chloroform-*d*)

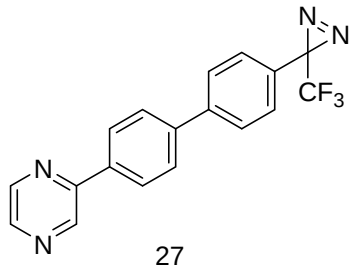


27

^{19}F NMR (470 MHz, Chloroform-*d*)

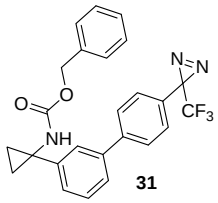


^{13}C NMR (126 MHz, Chloroform-*d*)



27

^{1}H NMR (500 MHz, Chloroform-*d*)



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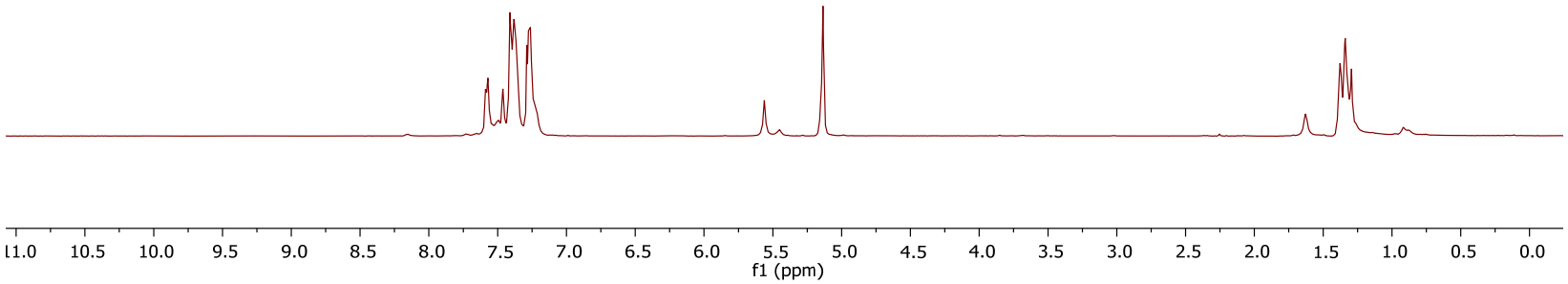
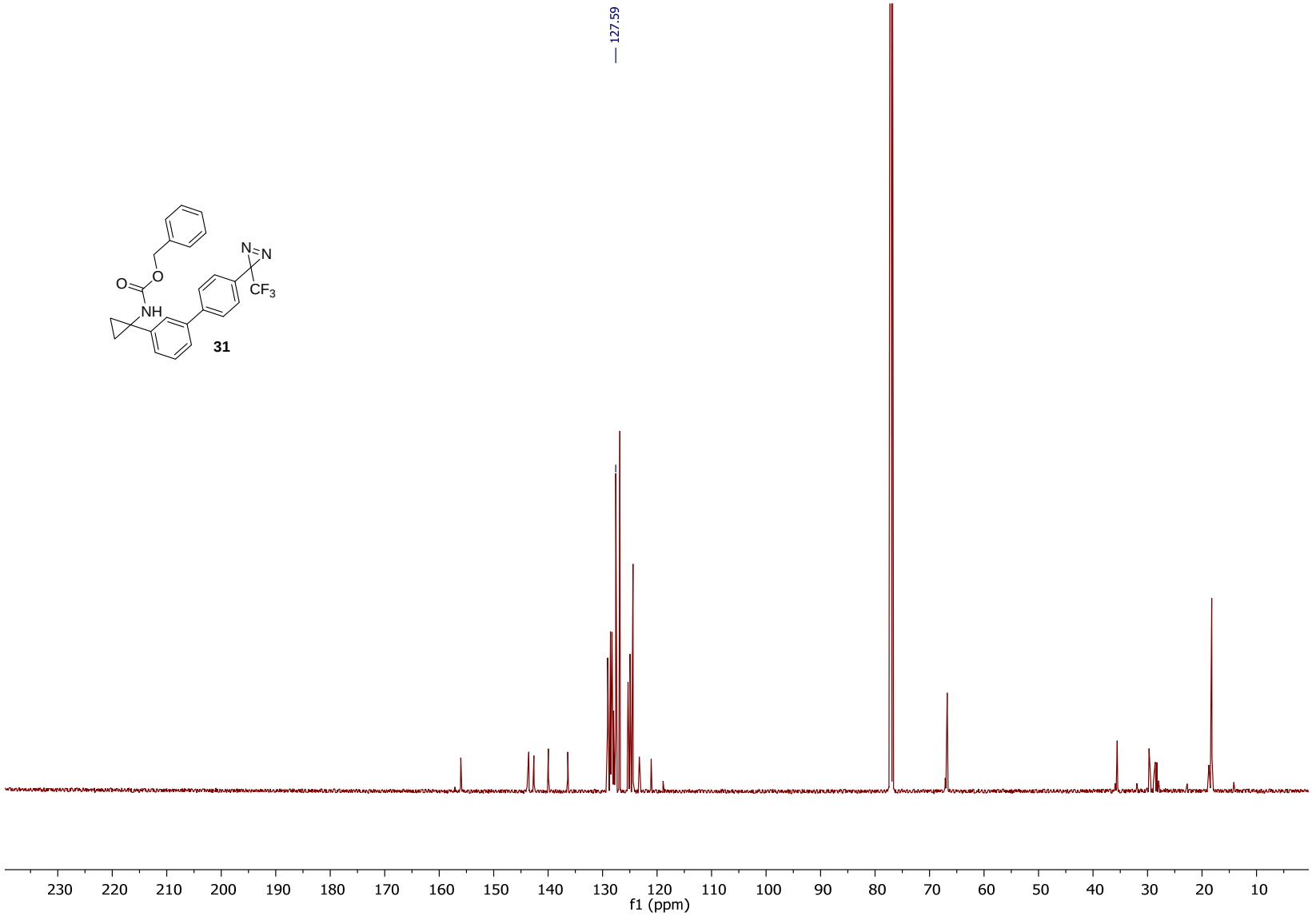
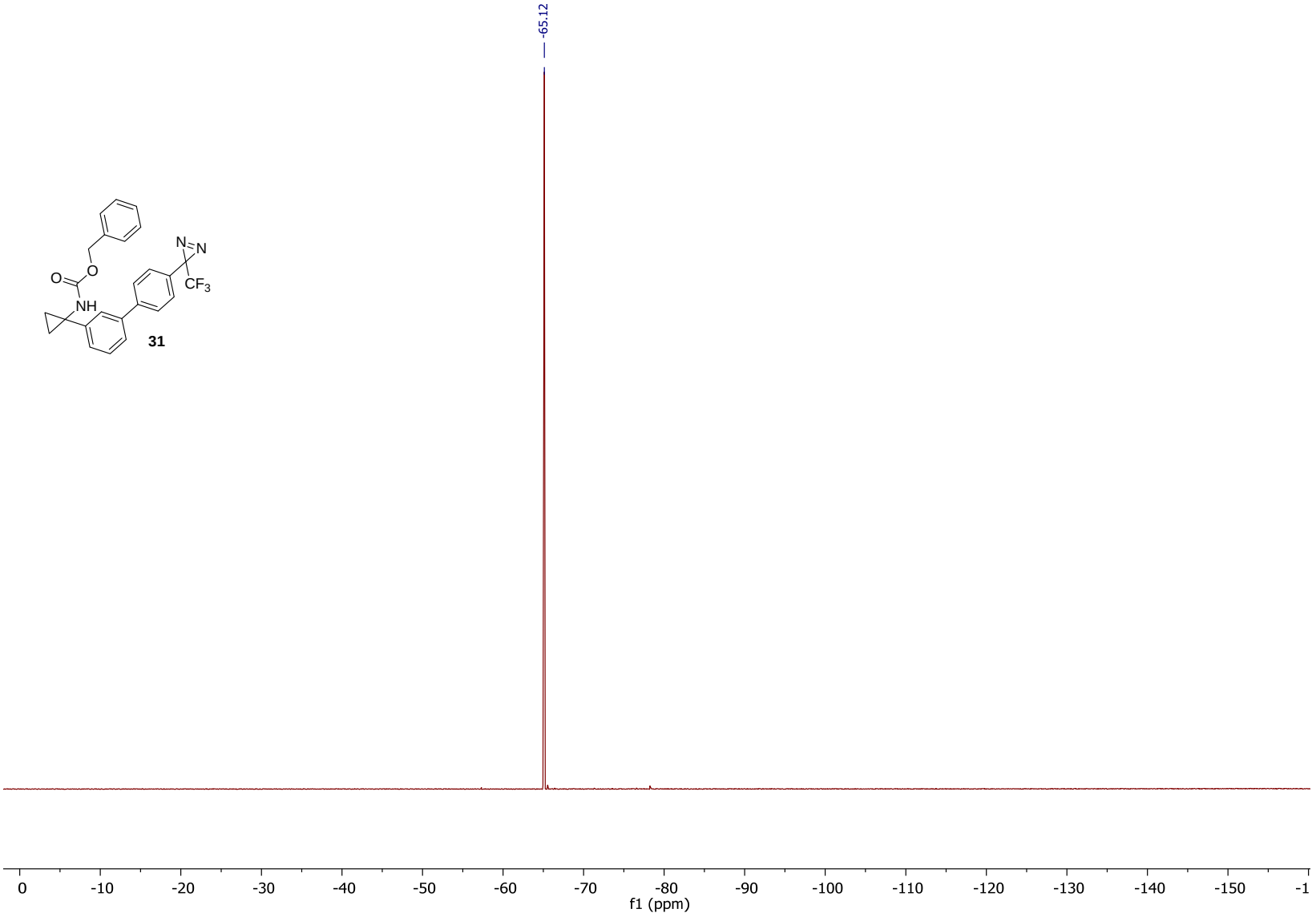


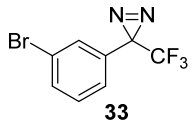
^{13}C NMR (126 MHz, Chloroform-*d*)



^{19}F NMR (471 MHz, Chloroform-*d*).



^{1}H NMR (500 MHz, Chloroform-*d*)



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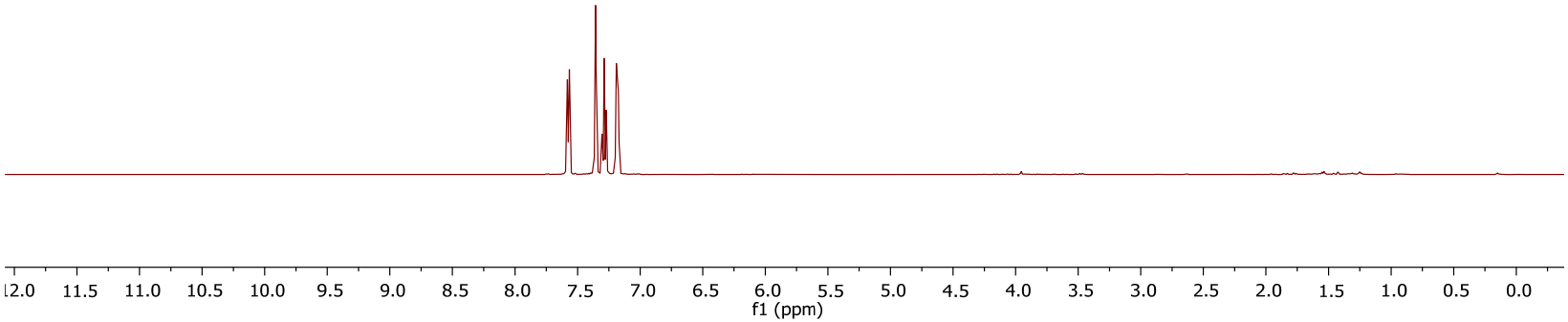
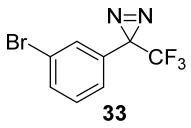
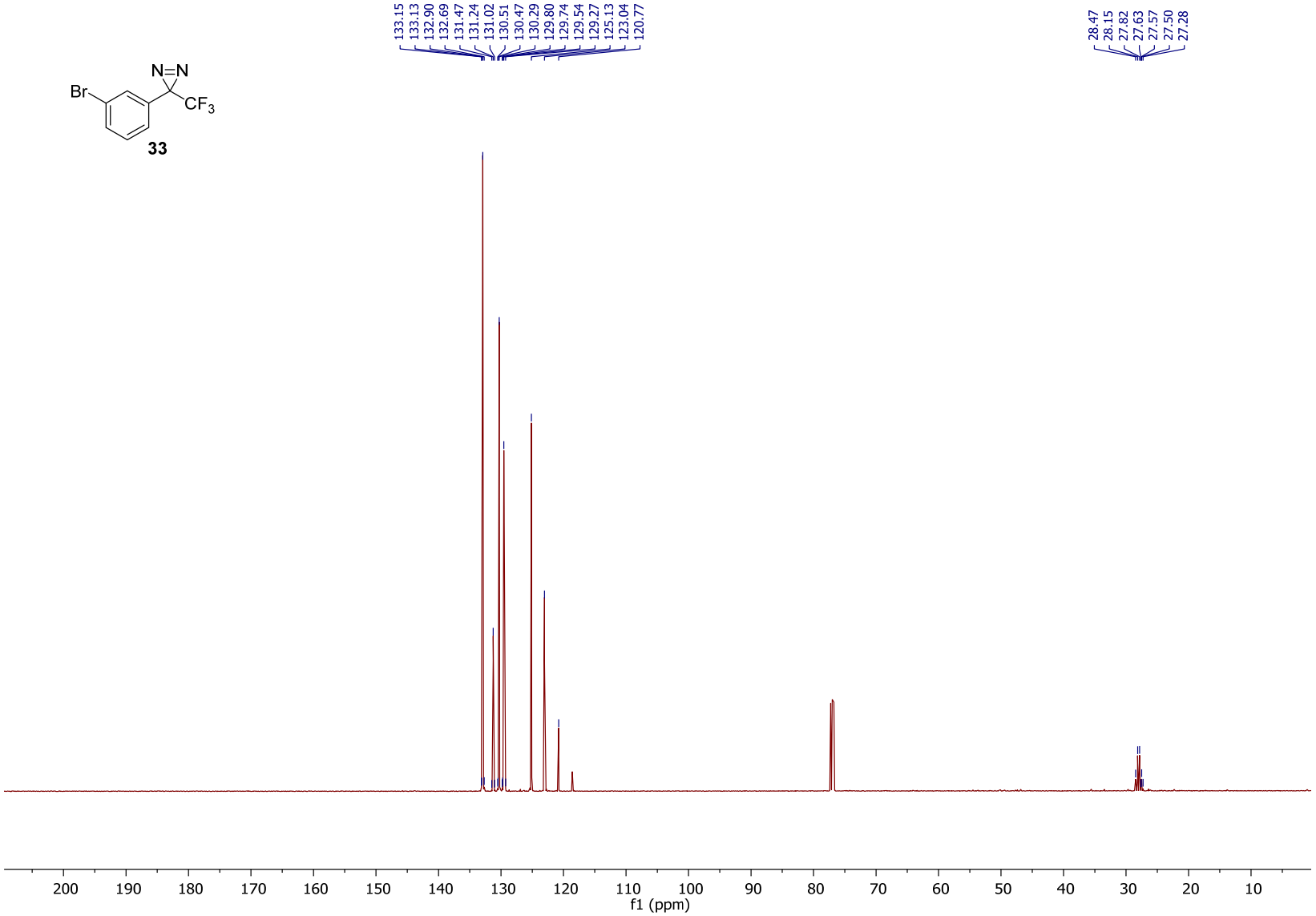


^{19}F NMR (471 MHz, Chloroform-*d*).

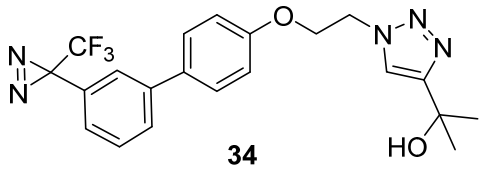


33

^{13}C NMR (126 MHz, Chloroform-*d*)



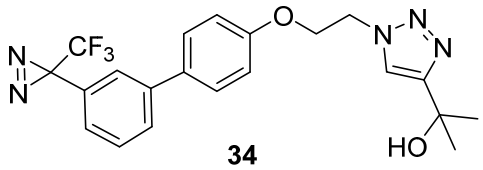
^{1}H NMR (500 MHz, Chloroform-*d*)



34

Product isolated as an inseparable mixture
b = peaks from boronate ester
e = ethyl acetate

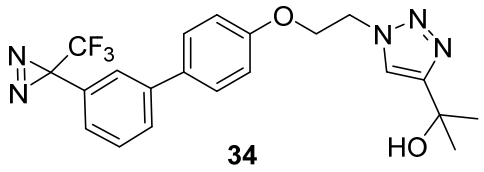
^{13}C NMR (126 MHz, Chloroform-*d*)



34

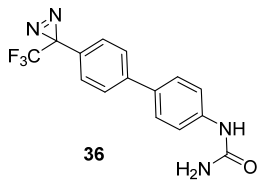
Product isolated as an inseparable mixture
p = peaks from product **34**
b = peaks from boronate ester
e = ethyl acetate

^{19}F NMR (470 MHz, Chloroform-*d*)



34

^{1}H NMR (500 MHz, DMSO-d_6)



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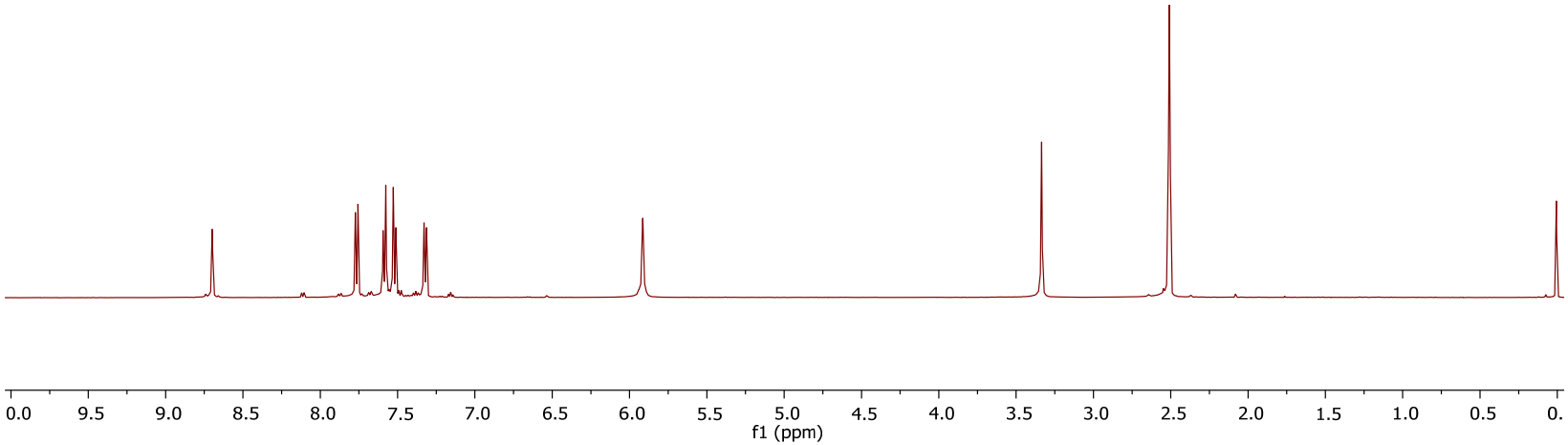
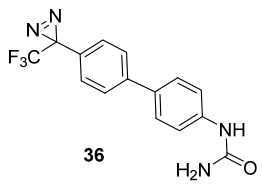


^{19}F NMR (471 MHz, DMSO-d_6)



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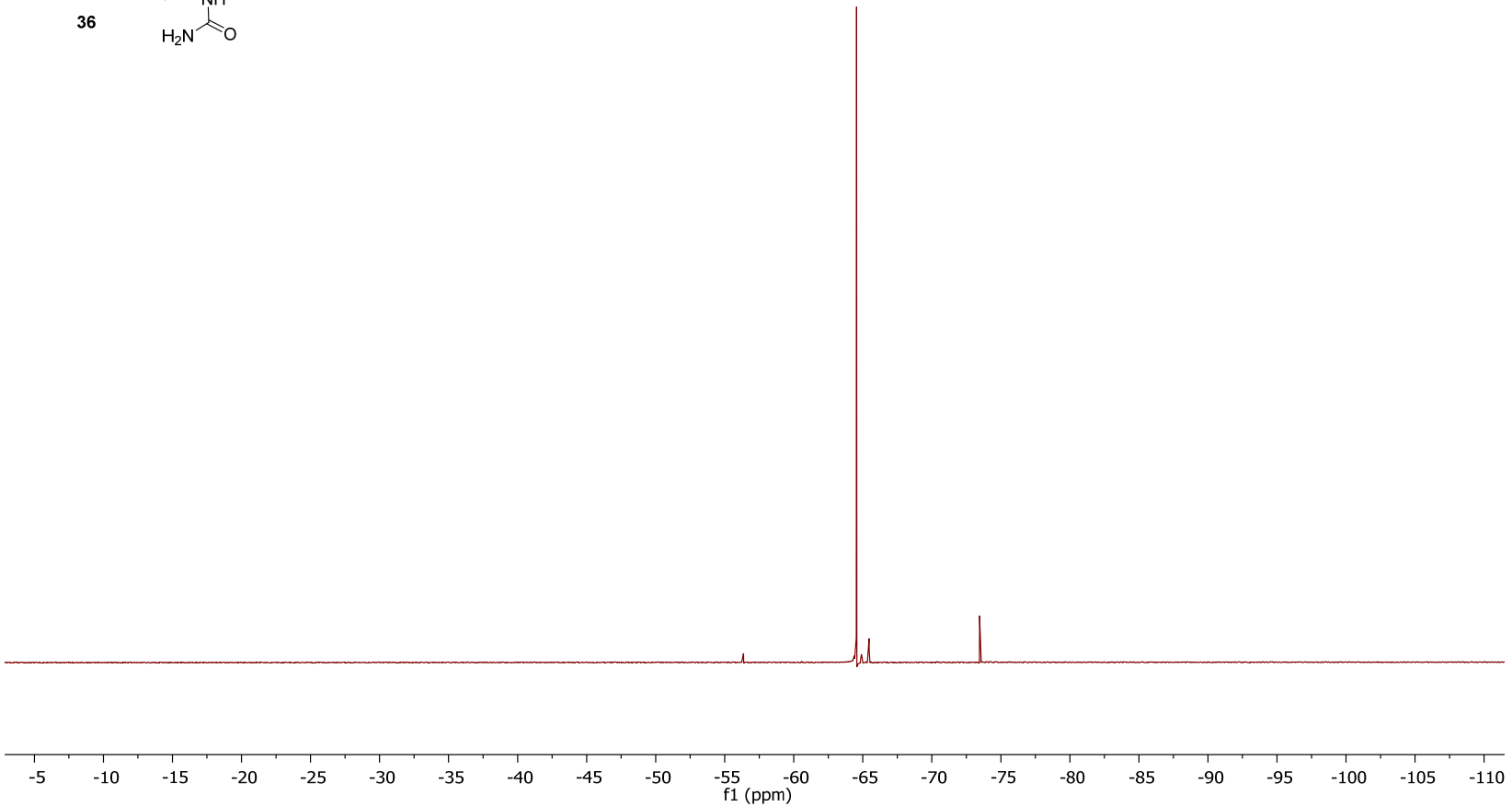
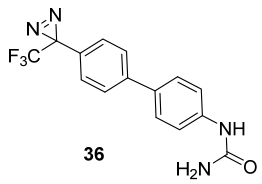


^{13}C NMR (126 MHz, DMSO-d_6)



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