Investigation of Small Molecule STAT3 Inhibitors

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ABSTRACT

STAT3 protein is crucial in cell proliferation, apoptosis, and host immune responses. In normal cell cycles STAT3's activation is fleeting, but in many cancer cell lines aberrantly activated STAT3 promotes cancer progression. The inhibition of STAT3 protein's ability to bind to DNA is an attractive treatment for aggressive carcinomas. The Tius Group has designed and synthesized a promising line of STAT3 inhibitors. The high activity of these inhibitors is attributable to the inclusion of a pentafluorobenzenesulfonamide or difluorocyanobenzesulfonamide moiety. A crucial fluorine atom in these inhibitors undergoes S_NAr with a cysteine residue in the protein.

In this thesis a small library of thirteen compounds and their SAR is reported. The difluorocyanobenzenesulfonamide "warhead" that was often included in inhibitors due to its potency, was found to be unstable in plasma due to reaction with native nucleophiles such as glutathione and plasma proteins. This provided the impetus to explore other scaffolds to attenuate the reactivity of the difluorocyanobenzenesulfonamide that would have desirable reactivity and pharmacokinetics. The SAR analysis of the "warhead" of the inhibitors is the focus of the work done. To attenuate the reactivity of the difluorocyanobenzenesulfonamide, inhibitors that incorporated constitutional isomers of the difluorocyanobenzesulfonamide: **S3I-H265**, **S3I-H270**, **S3I-H275**, and **S3I-H289** were made. They indicated through their potency and lack of plasma stability that they would be unsuitable for further study. Inhibitors **S3I-H254**, **S3I-H261** replaced the fluorines in the difluorocyanobenzenesulfonamide motif with a chlorine one at a time. Inhibitors **S3I-H296**, **S3I-H299**, and **S3I-H308** were made in order to determine whether the substitution of an amide for the nitrile group would lead to higher potency and stability, however, the potency of these inhibitors was low. To explore whether alkyl substitution would improve the qualities of the pentafluorobenzenesulfonamide **S3I-H287** was made. The activity of this compound was only slightly higher than that of **S3I-H247** its parent. The activity of the inhibitors synthesized for this thesis indicate that novel reactive scaffolds need to be explored in order to provide a candidate for clinical trials.

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List of Synthetic Schemes

List of Abbreviations

Ar	Argon
Arg	Arginine
Boc	*Tert*-butyloxycarbonyl
δ (ppm)	Chemical Shift (parts per million)
Cat.	Catalytic
CCD	Coil Coil Domain
^{o}C	Degrees Celsius
DBD	DNA Binding Domain
DMA	Dimethylamine
EMSA	Electrophilic Mobility Shift Assay
Eq.	Equivalents
ESI	Electrospray Ionization
G	Grams
Gly	Glycine
h	Hours
HLM	Human Liver Microsomes
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrum
IC_{50}	Half Maximal Inhibitory Concentration
J	Coupling Constant
JAK	Janus Kinases
LC-MS TOF	Liquid Chromatography-mass Spectrometry Time-of-flight
Lys	Lysine
µM	Micromolar
µmol	Micromoles
M	Molar
m/z	Mass-to-charge Ratio

M+	Molecular Ion
mg	Milligram(s)
MHz	Megahertz
min	Minute(s)
mL	Milliliter
MLM	Mouse Liver Microsomes
Mmol	Millimolar
ND	N-terminal Domain
nm	Nanometer(s)
NMR	Nuclear Magnetic Resonance
Papp	Apparent Permeability Coefficient
PBS	Phosphate-buffered Saline
%	Percent
PTLC	Preparative Thin Layer Chromatography
sec	Second(s)
SAR	Structure-activity Relationship
Sat	Saturated
SH2	Src Homology 2
SIF	Simulated Intestinal Fluid
S_N2	Nucleophilic Substitution, Second Order
S_NAr	Nucleophilic Aromatic Substitution
SPhos	2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl
STAT3	Signal Transducer and Activator of Transcription 3
t-/tert-	tertiary
$t_{1/2}$	Half-life

TAD	Transactivation Domain
TLC	Thin Layer Chromatography
UH	University of Hawai'i
Xantphos	4,5-(bisdiphenylphosphino)-9,9-dimethylxanthene

Chapter 1:

History of and Progress Towards a Clinical Candidate for STAT3 Inhibition

The signal transducer and activation of transcription (STAT) protein family consists of seven members: STAT 1, 2, 3, 4, 5A, 5B, and 6. These proteins are responsible for a variety of functions in cells such as inflammation, proliferation, growth, differentiation, and apoptosis. This family of proteins each contain five homologous domains: the N-terminal, coiled-coil, DNA-binding, SRC homology 2 (SH2), and the transactivation domains.[1] Another important shared feature by this group of proteins is an essential tyrosine (Tyr) residue located in the C-terminus (Tyr705 in STAT3) that is phosphorylated during normal activation.[2]

The pathway for activation of STAT3 is intertwined with janus kinases (JAK). Janus kinases, named for the two faced Roman god Janus due to their ability to have both mitotic and anti-mitotic effects on the cell cycle, play a crucial role in STAT3 activation.[3] The pathway begins with cytokines such as interleukin (IL-6) binding to receptor proteins. Protein binding results in conformational changes that displace JAK. The kinases are then free to phosphorylate STAT3 at Tyr705. Upon phosphorylation STAT3 is free to bind to the SH2 domain of another STAT protein. This creates a stable dimeric structure that is able to translocate to the nucleus of the cell where it may affect the cell cycle.[4] In healthy cell lines the STAT proteins' fleeting activation encourages a regular rate of division, growth, and immune responses.[5,6]

STAT3 has an intimate role in the inflammation response associated with many types of basic injuries. Keratinocytes with low levels of STAT3 have poor wound healing responses *in vivo* and *in vitro*, attributable to the lack of cellular migration.[7] Additionally, STAT3 is critical in the body's immune response to chronic arthritis, psoriasis, Crohn's disease, and cancers.[8, 9, 10]

Up regulation of STAT proteins, specifically STAT3, is associated with proliferation in many types of cancer cell lines. STAT3 is aberrantly activated in at least eighteen different types of cancers including lung, breast, prostate, colon, melanoma, various leukemias, and gastric (**Figure 1**).[11-16] Loss of function of STAT3 in cancerous cell lines leads to a disruption of the cell cycle, cellular movement, and potentially apoptosis. While it is important to regulate the formation of STAT3:STAT3 dimer to limit the progression of cancer, in many cancer lines it is undesirable to inhibit the formation of STAT1:STAT1 and STAT5:STAT5 dimer. These proteins heighten immune response in the tumor microenvironment when functioning normally.[17] The

selective inhibition of aberrantly activated STAT3 is thus a desirable outcome in the treatment of aggressive carcinomas.[18]

Tumour type	Activated STAT factor
Multiple myeloma	STAT3
Acute lymphocytic leukemia (ALL)	STATI, STAT5
Chronic lymphocytic leukemia (CLL)	STATI, STAT3
Acute myelogenous leukemia (AML)	STATI, STAT3, STAT5
Large granular lymphocyte leukemia (LGL)	STAT3
Chronic myelogenous leukemia	STAT5
Lung cancer	STAT3
Breast cancer	STAT3
Renal cancer	STAT3
Prostate cancer	STAT3
Pancreatic carcinoma	STAT3
Melanoma	STAT3
Colon carcinoma	STAT3
Gastric carcinoma	STAT3
Cervical cancer	STAT3
Ovarian cancer	STAT3
Hepatocellular carcinoma	STAT3
Head and neck cancers	STAT3

Figure 1. Known examples of cancers that have aberrantly activated STAT3.[19]

The types of inhibitors that have been explored to date are: natural products, tyrosine kinase inhibitors, protein based inhibitors, and small molecule non-protein based inhibitors.[7] The antitumor activity of some natural products has recently been explained through a mechanism of action involving regulation of STAT3 and the JAK signaling pathway. These pathways are activated in response to injury and many diseases in order to save host cells from the immune response to inflammation. This same mechanism is used in cancerous cell lines to survive the immune response of the host. Traditional medicines administered as salves, pastes, and tinctures of plant extracts contain active ingredients such as celastrol, curcumin, resveratrol, cucurbitacins, capsaicin, and sanguarine (**Figure 2**).[19-24] The wide variety of illnesses such as asthma, skin conditions, diabetes, obesity, wounds, arthritis, and cancers targeted by these medicinal treatments shows the wide role that STAT3 plays in the regulation of immune responses and inflammation.

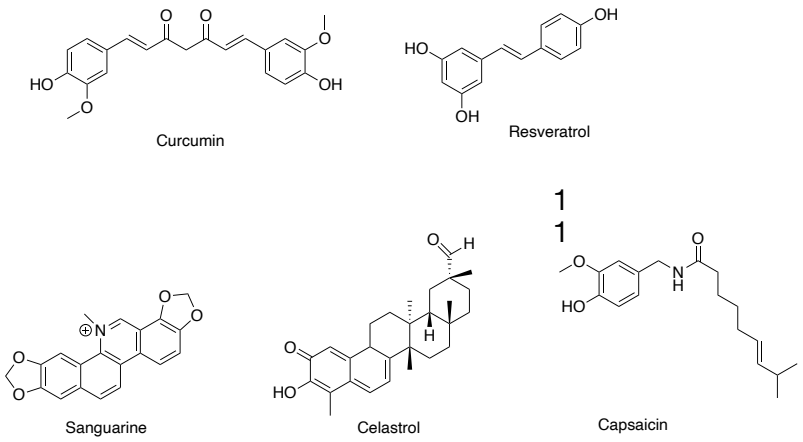


Figure 2. Natural products that inhibit STAT3.

Despite the micromolar potency of the above mentioned natural products there have been few examples of these compounds being used as a starting point for drug development because in many cases the specific mechanism of action on the JAK signaling pathway is not yet fully understood. In the 1990s pharmaceutical companies became interested in developing treatments to target proteins and kinases associated with cancer progression as various cellular signaling pathways began to be defined, such as the JAK pathway. The development of kinase inhibitors began to bloom in 2001 with the first FDA approval of a targeted kinase inhibitor, imatinib.[25] Since then targeting kinases has become one of the most studied strategies for cancer treatment. This is because of the ability of kinase inhibitors to be used in tandem with other treatment options and the notable selectivity for their targets.

Protein based inhibitors have attempted to target inhibition of dimerization in the STAT3 SH2 domain, but this is a challenge due to the impracticality of designing a sequence of amino acids that is cell permeable, and able to reach the druggable pocket of the protein. Many *in vitro* studies have indicated protein based kinase inhibitors are able to induce apoptosis in a variety of cancer cell lines including breast, prostate, non-small cell lung cancer, and pancreatic.[26,27] However, many of the protein based inhibitors reported exhibit problems with stability, cell

permeability, and poor pharmacokinetics. These factors have curtailed extensive research into the development of novel protein-based therapeutics for STAT3 inhibition.

The work that will be described in this thesis falls under the class of STAT3 inhibitors that were mentioned last: small-molecule non-protein based inhibitors. Small-molecule non-protein based inhibitors show promise in selectively inhibiting aberrantly activated STAT3 in cancer cell lines. The compounds disclosed in this thesis are orally bioavailable, potent, selective for STAT3 inhibition even relative to other members of the STAT family, and display limited cytotoxicity to healthy cell lines.[28] The work in this thesis takes on the challenge of targeting protein-protein interaction. The targeting of protein-protein interaction is a challenge for several reasons. The first reason is that proteins must have suitable structural folding that exposes amino acids that enable non covalent and covalent interactions with the drug. The only opportunity a drug has to be effective is in the druggable pocket of the desired protein. This challenge is increased by the fact that in many cases the pocket that is available is not typically well suited to high binding affinity.[29] This limits the number of afflictions which are able to be treated by this strategy. The molecules that were targeted in this work inhibit both the dimerization of STAT3 and its ability to bind to DNA .

An attractive way to target the STAT3 protein is prior to dimerization in the cytoplasm and the subsequent translocation to the nucleus.[7] One way to achieve this is to prevent the protein from being phosphorylated by the JAK which allows it to make the active STAT3:STAT3 dimer. A variety of small molecule non-protein based STAT3 dimerization inhibitors have consequently been the target of research for quite some time. Identification of the original lead compound, **S3I-201**, by *in silico* screening of the National Cancer Institute (NCI) database by Turkson in 2007 began research into a promising line of STAT3 inhibitors (**Figure** 4).[2] **S3I-201** selectively inhibits the SH2 domain of STAT3 and has an IC_{50} value of 86 μM ± 33 μM. In mouse xenografts (MDA-MB-231), tumor growth was significantly inhibited upon the I.V. injection of **S3I-201** (**Figure 3**). Notably, **S3I-201** was later shown to interact with two of three pockets in the SH2 domain of STAT3. A key interaction which exploited hydrophobic interactions of the SH2 domain was not present in this early lead compound.

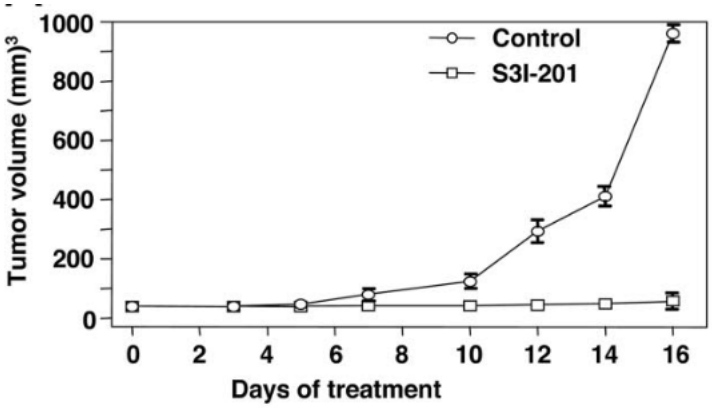


Figure 3. Tumor volume vs. days of treatment with **S3I-201** (5 mg/kg) by I.V. every 2/3 days.[2]

Computer optimization studies developed the next lead compound, **S3I-201.1066** (IC_{50} 35 μM), by Turkson and coworkers.[30] The compound exhibited superior potency and computer modeling showed that the compound fits into pockets of the SH2 domain of STAT3. Fitting into the third pocket exploits hydrophobic interactions from Phe716, Ile659, Val637, Trp623 (**Figure 4**). Similar to its predecessor, treatment of mice xenografts (MDA-MB-231) with 2-3 mg/kg of **S3I-201.1066** by I.V. every two to three days for 17 days led to reduction of tumor volume with no signs of toxicity to the mouse host (**Figure 5**).

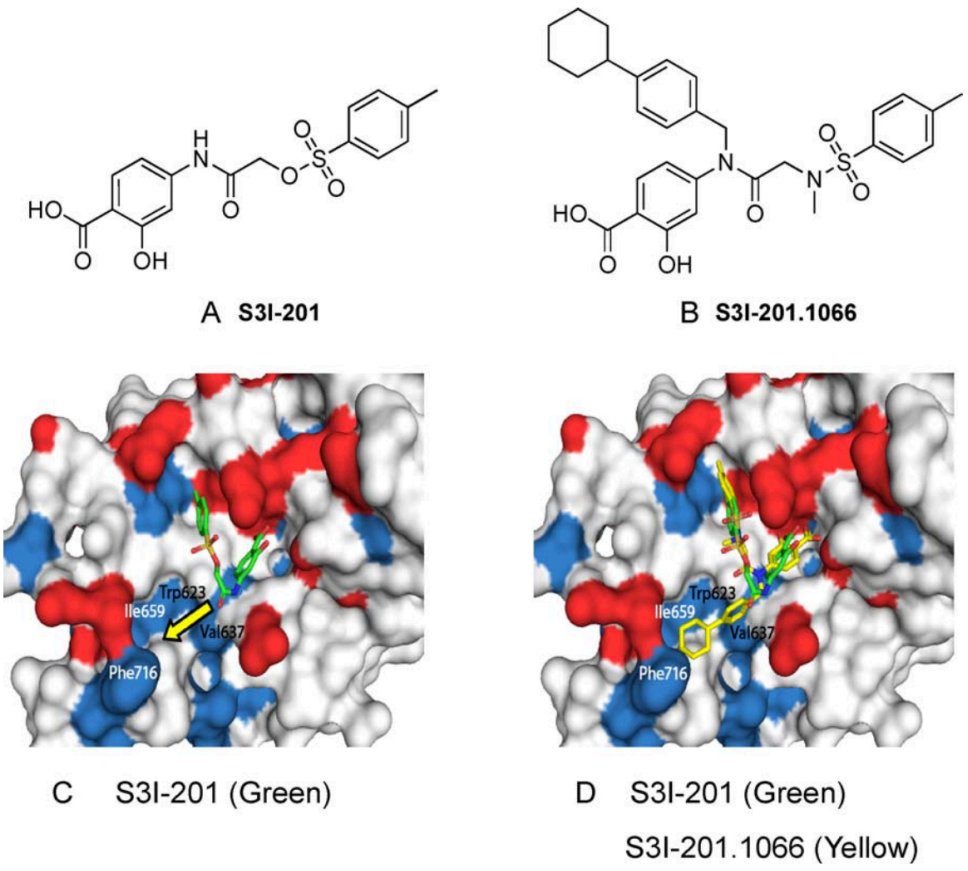


Figure 4. Structures and interactions of the novel leads in the SH2 domain of STAT3.[30]

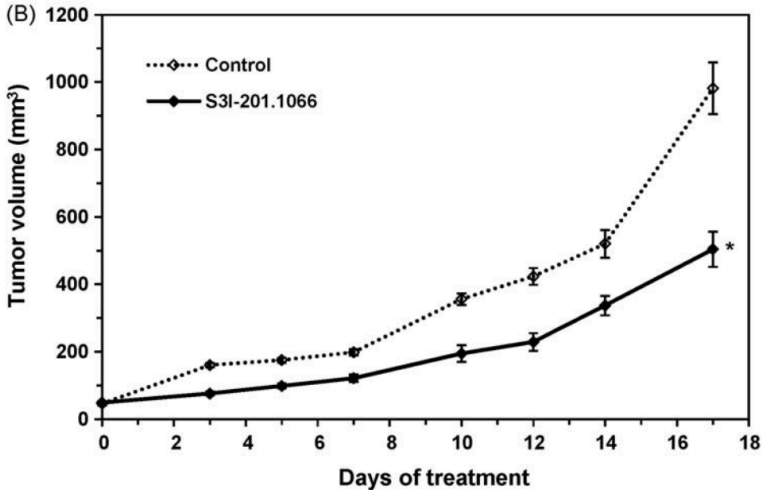


Figure 5. Results of I.V. injection of mice containing human breast tumors with **S3I-201.1066**. [30]

The next promising discovery of a STAT3 inhibitor was **BP-1-102** by Turkson and Gunning.[28] The new lead compound contained a promising pentafluorobenzene sulfonamide moiety that improved potency in the electrophoretic mobility shift assay (EMSA; IC_{50} 6.8 μM). **BP-1-102** also showed significant *in vitro* activity (IC_{50} 10-20 μM). Importantly, STAT3:STAT3 dimers were targeted selectively. STAT1:STAT1 and STAT5:STAT5 dimer formation was not inhibited. The increased activity can be attributed in part to the pentafluorobenzene sulfonamide moiety positioned into the SH2 pocket containing Lys591, Glu594, Ile634, Arg595. **BP-1-102** interacts with three significant sub-pockets associated with the SH2 domain (**Figure 6**). The pentafluorobenzene sulfonamide exploits hydrophobic interactions in the protein, and may engage in hydrogen bonding.

Notably, **BP-1-102** was the first orally bioavailable STAT3 inhibitor disclosed. It was administered via oral gavage as well as I.V. in mice at 3 mg/kg. Significantly, there was little difference in the amount of tumor tissue present in hosts that were given the drug orally or intravenously. The mice did not show any signs of acute toxicity such as loss of appetite, lethargy, weight loss, or gross alteration of internal organs over the administration period.

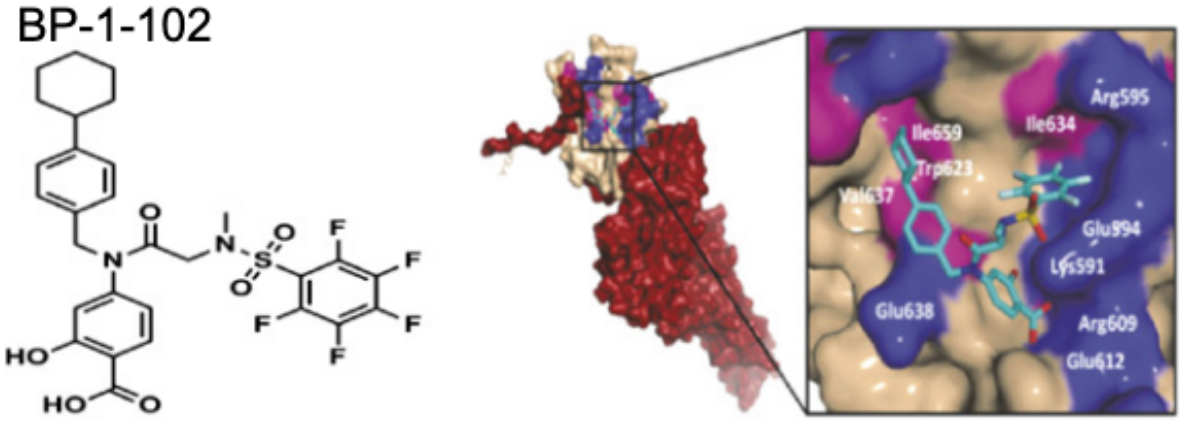
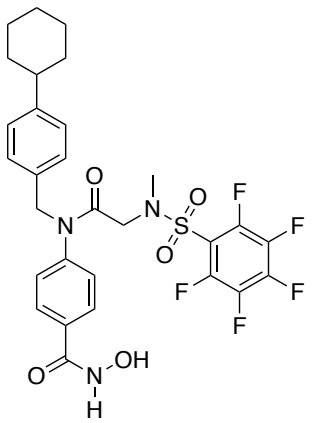


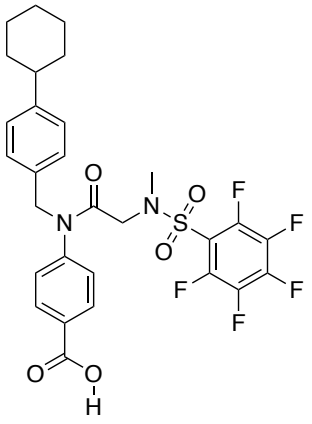
Figure 6. The structure of **BP-1-102** along with a computer simulation of binding in the SH2 domain of STAT3.[28]

Years later, following collaboration with the Tius Group, Turkson disclosed two new lead compounds **SH5-07** (IC_{50} 3.9 μM) and **SH4-54** (IC_{50} 4.6 μM).[31] The first compound discovered was a hydroxamic acid derivative of the previous lead **BP-1-102**. The other novel compound discovered was a benzoic acid derivative of **BP-1-102** (**Figure 7**). These new lead compounds showed notable increases in potency compared to **BP-1-102**.

The inhibition potency of this line of small molecules is not necessarily correlated with their ability to inhibit tyrosine phosphorylation. The increase of **SH5-07** and **SH4-54's** potency was explained following the observation that intracellular active STAT3 (pY703STAT3) DNA-binding decreased with no corresponding change of pY703STAT3 and there was still inhibition of binding of STAT3 to DNA. This suggested that the inhibition is strongest on the DNA-binding activity of STAT3. At concentrations in the 1-3 μM range, compounds did not induce any changes in pY703STAT3 and inhibition of phosphorylation was only seen at the 5-10 μM range. The non-linear relationship of constitutively active STAT3 suggests that the pre-existing STAT3:STAT3 dimer's ability to bind to DNA is also being inhibited. Affinity at the SH2 and DBD is responsible for the increase in potency of **SH5-07** and **SH4-54**. The two compounds' activity at the DBD is hypothesized to arise from the fluorine para to the sulfonamide interacting irreversibly with a cysteine residue via nucleophilic aromatic substitution reaction (S_NAr).



SH5-07



SH4-54

Figure 7. Shows the structures of **SH5-07** and **SH4-54** respectively.

The amount of work done for this project was too much to have been accomplished by one person. As such, several members of the Tius Group worked on developing new compounds. My part in this work was to investigate the SAR of R_4. This part of the molecule is crucial to the S_NAr reactivity of the inhibitors at a cysteine residue in the DBD of STAT3. Understanding the SAR of R_4 is crucial to producing new inhibitors. As results from each target came in, they provided insight into how this part of the compound affects the reactivity of the inhibitors. This guided us the design of subsequent inhibitors.

The high potency along with high selectivity for STAT3:STAT3 dimer shown by **SH5-07** and **SH4-54** offered an opportunity to explore the structure-activity relationships (SAR) of related small molecules in order to discover compounds with improved drug-like properties. Electrophoretic mobility assays performed to interrogate the selectivity of the novel compounds indicated that they targeted STAT3:STAT3 dimerization nearly exclusively and did not significantly inhibit STAT1:STAT1 or STAT5:STAT5 dimerization. In order to effectively prepare compounds of promise a general scaffold for future STAT3 inhibitors was defined (**Figure 8**). This consists of four regions (R_1, R_2, R_3, R_4) to vary, as well as a linker region.

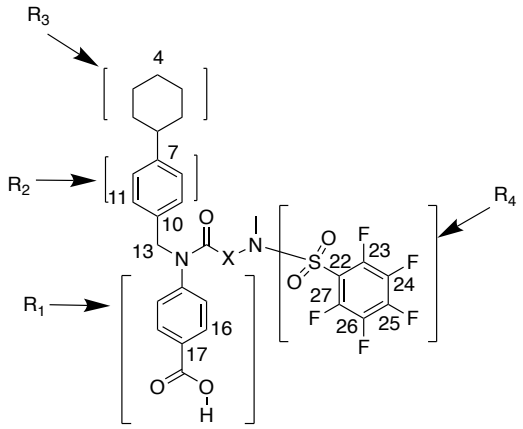


Figure 8. General regions which can be varied to explore the SAR of the inhibitors, as well as a numbering scheme for the carbon atoms.

The first region that was optimized for potency, permeability, and stability was the linker region (X). Replacement of the Gly-linker used in **BP-1-102** with Ala-(X=CHMe), led to (*R*)-**S3I-H012** (IC_{50} 3.0 μM). The corresponding (*S*)-enantiomer **S3I-H011** (IC_{50} 5.0 μM) has potency notably lower than its antipode (**Table 1**). Interestingly, substituting Ala-based linkers for the Gly-based linker used in **SH4-57** to make (*R*)-**S3I-H015** (IC_{50} 9.3 μM) and (*S*)-**S3I-H016** (IC_{50} 10.0μM) led to a decrease in potency. Substituting Ala-based linkers from the Gly-based linkers in lead compound **SH5-07** (IC_{50} 4.4 μM) gave (*R*)-**S3I-H017 (**IC_{50} 5.3 μM) and (*S*)-**S3I-H018** (IC_{50} 6.8 μM), the (*R*) enantiomer once again had superior potency. The replacement of the Gly-based linker of **BP-1-102** with $CH(CH_2CH_3)$ gave compounds (*R*)-**S3I-H022** (IC_{50} 6.9 μM) and (*S*)-**S3I-H021** (IC_{50} 10 μM). To fully explore alterations in the linker region compounds that included X= cyclopropyl, $C(CH_3)_2$, Ser-based linkers, hydroxyethyl, Thr-based linkers, aminomethyl, and an Asp-based linker were all synthesized. In all cases the (*R*) enantiomers were at least as potent as their (*S*) antipodes. From this juncture on only (*R*) enantiomers were used in SAR studies.

Table 1. SAR Analysis of Linker Region.

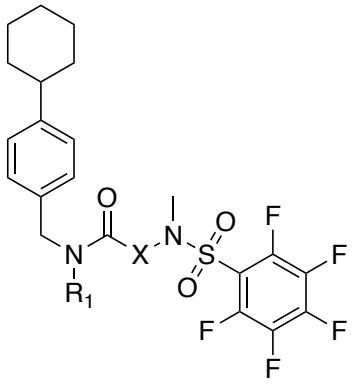
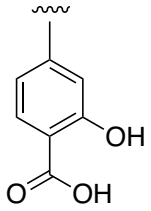
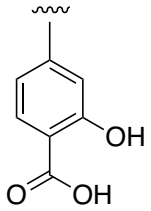
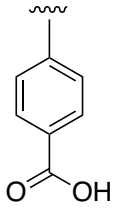
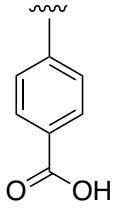
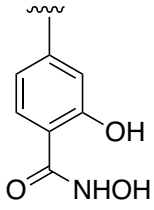
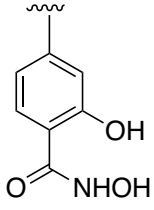
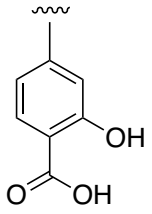
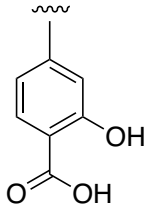
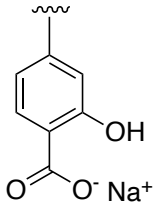


Table 1

Compound	R_1	Linker (X)	IC_{50} µM
S3I-H012		(*R*)-CHMe	3
S3I-H011		(*S*)-CHMe	5
S3I-H015		(*R*)-CHMe	9.3
S3I-H016		(*S*)-CHMe	10
S3I-H017		(*R*)-CHMe	5.3
S3I-H018		(*S*)-CHMe	6.8

Compound	R_1	Linker (X)	IC_{50} µM
S3I-H022		(*R*)-CH(CH_2CH_3)	6.9
S3I-H021		(*S*)-CH(CH_2CH_3)	10
S3I-H048		(*R*)-CHMe	6.4

When the Gly-based linker of **BP-1-102** was replaced with alanine the potency increased, but the metabolic stability decreased markedly. There was a reduction in stability noted in compound **S3I-H048** in mouse liver microsomes/human liver microsomes (MLM/HLM) $t_{1/2}$ = 5 min vs **SH4-54** $t_{1/2}$= 17 min that ended the Ala-based linker's future use (**Table 1**). In an effort to maintain the activity of the Ala-based linkers but increase metabolic stability, *(R)*-proline based linkers were used in its place. Changing the Ala-based linker in **S3I-H048** to a proline-based linker provided **S3I-H070** (IC_{50} 2.1 ± 0.2 µM) which had an increase in potency (**Figure 9**). Satisfyingly, **S3I-H070** was metabolically stable. When replacing the proline-based linker with an *(R)*-azetidine linker to yield **S3I-H098** (IC_{50} 0.54 ± 0.01 µM) a further increase in potency was observed. This significant increase in potency and the adequate metabolic stability of the *(R)*-azetidine linker led to its exclusive use in SAR studies from this point on.

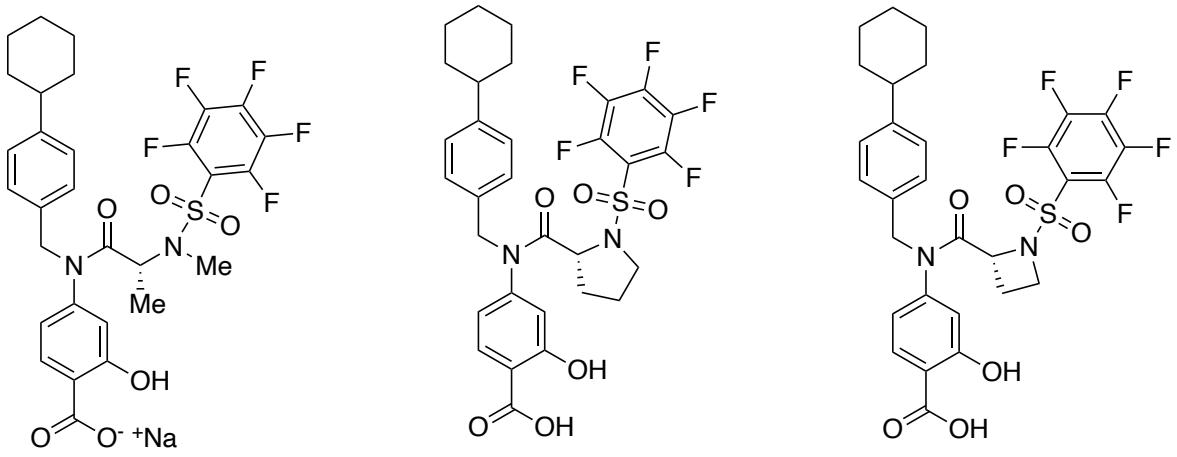
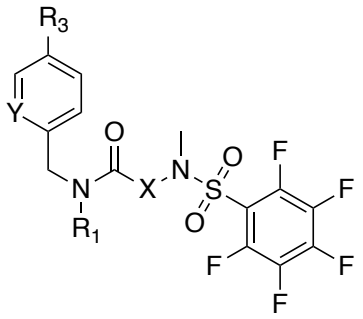


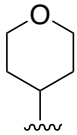
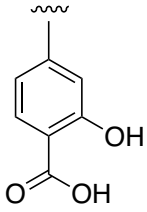
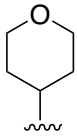
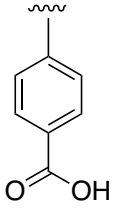
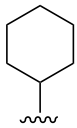
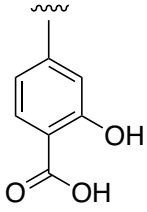
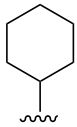
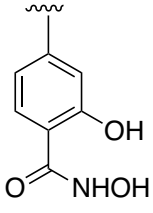
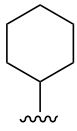
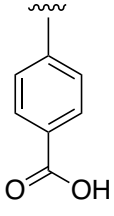
Figure 9. The structure of **S3I-H048**, **S3I-H070**, and **S3I-H098** respectively

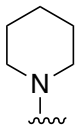
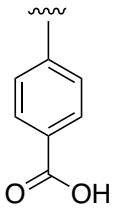
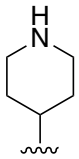
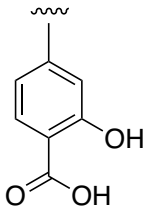
The hydrophobic region of the compounds was also the subject of extensive SAR studies. Variation of the R_3 group to THP, cyclopentyl, 4-piperdinyl, (4,4)-difluorocyclohexyl, cycloheptyl, and various others provided compounds that exhibited comparable, or greater EMSA potency than **BP-1-102**. However, compounds which lacked a cyclohexyl group had a decrease in potency in MDA-231 human breast cancer cell lines (**Table 2**). The compounds containing a THP group in R_3 (**S3I-H115**; **S3I-H124**) were completely inactive in MDA-231 cells, while compounds (**SH5-07**; **SH4-54**) with a cyclohexyl group had comparable potency in MDA-231 cells. Compounds that had a nitrogen in $R_{3,}$, such as, **S3I-H020** and **S3I-H039** were not potent enough to warrant testing in cellular assays. It is hypothesized that these results are reflective of the fact that in order to permeate cells the cyclohexyl group is necessary.

It is noteworthy that the EMSA values of all compounds are artificially low. This is because of the dithiothreitol that is used in the EMSA analysis. The dithiothreitol is used as a reducing agent in order to prevent cysteine residues in the protein from forming disulfide bonds. The sulphur in the stabilizer acts as a nucleophile that can participate in S_NAr with the inhibitors. This lowers the concentration of the drug available to inhibit STAT3 protein which results in artificially low EMSA values. The assays still serve as a reliable way to make comparisons of potency from compound to compound.

Table 2. Selected changes in R_3 which interacts with the hydrophobic pocket of the SH2 domain.



Compound	R_3	R_1	X	Y	IC_{50} µM	MDA-231,72h,IC_{50}
S3I-H115			(*R*)-Azetidine	CH	1.09	Inactive
S3I-H124			(*R*)-Azetidine	CH	3.94	Inactive
BP-1-102			CH_2	CH	6.8	10-20 µM
SH5-07			CH_2	CH	3.9	1.8 µM
SH4-54			CH_2	CH	4.6	3.0 µM

Compound	R_3	R_1	X	Y	IC_{50} µM	MDA-231,72h,IC_{50}
S3I-H020			CH_2	CH	26.4	N/A
S3I-H039			CH_2	CH	30.1	N/A

Later SAR studies were done on the R_2 region. The phenyl group was replaced by a pyridine or a pyrazine (**Figure 10**). It was shown through the synthesis of a series of ten analogues that the pyridine containing compounds had higher potency than the compounds containing pyrazine. The compounds are shown in pairs, making the comparison of pyridine and pyrazine possible across four pairs of inhibitors. Although there is one outlier in the compound set (**S3I-H204**; **S3I-H203**), which presents the opportunity for future work, the combination of the cyclohexyl and pyridine units in R_3 and R_2, respectively, has been the focus of most of the SAR.

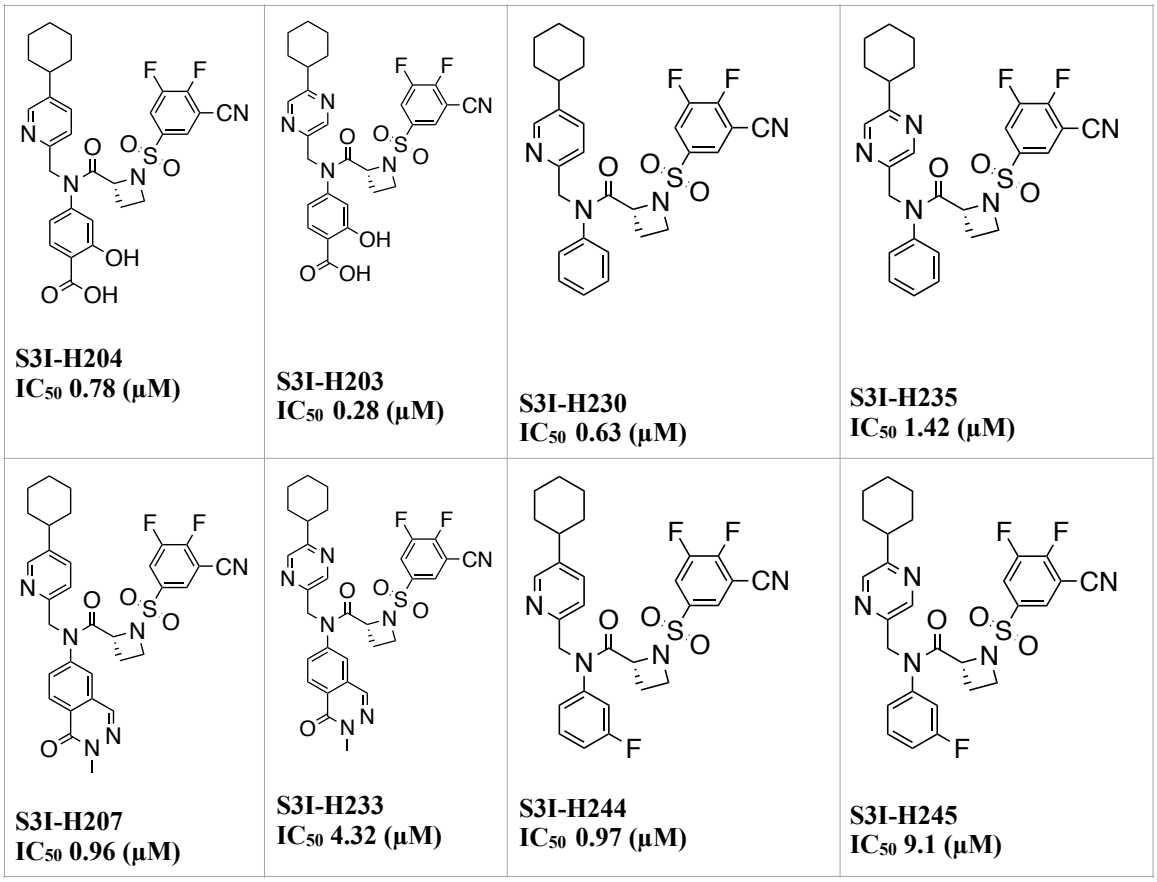
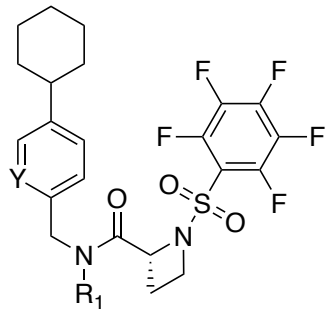


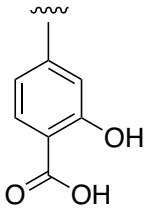
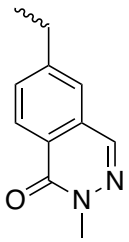
Figure 10. Potency and structures of a set of analogues meant to probe the SAR of R_2.

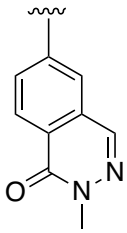
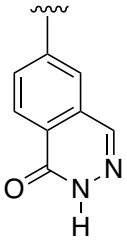
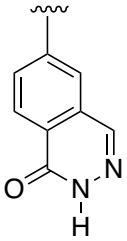
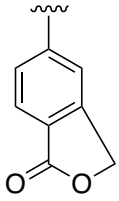
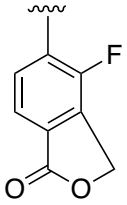
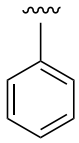
Region R_1 which consisted of a salicylic acid moiety in the first several lead compounds and that was originally thought to be necessary for maintaining potency in analogues has since been identified as problematic due to a lack of cell permeability. *In vitro* studies indicated that compounds are not cell permeable when functionalities such as the hydroxamic, salicylic, or carboxylic acid groups are present at C17 (**Figure 8**). Permeability studies were performed by biologists with Caco-2 cell lines to simulate intestinal uptake of these inhibitors. The Caco-2 cell lines are human colon carcinoma cells that are grown on permeable plastic supports in multi-welled plates. Compounds are then added to apical (cells of the monolayer facing the lumen) or basolateral (cells not facing the lumen) sides of the individual wells of the monolayer and the flux of the cells between the layers is then measured. In order to be considered to have acceptable permeability the desired apical to basolateral efflux should be >9.0 x 10^{-7} cm/s. These functional groups were deemed undesirable for inclusion in future targets.

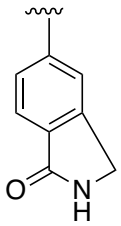
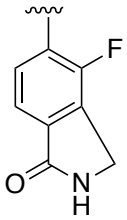
Extensive modifications to region R_1 have been made. Many of the first compounds represented small variations on the salicylic acid. Following results from cell permeability studies, the Tius Group began to experiment with other functional groups. The simplest group, a phenyl, was shown to have superior EMSA potency but stability studies seemed to indicate that the compounds are susceptible to oxidation by liver enzymes. Some of the new compounds contained fluorinated phenyls, aryl amides, and the phthalazinone motif in place of the salicylic acid (**Table 3**).

Table 3. SAR of region R_1.



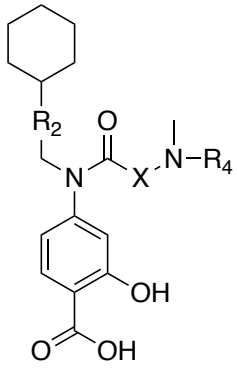
Compound	R_1	Y	IC_{50} µM
S3I-H127		N	0.38
S3I-H205		N	0.93

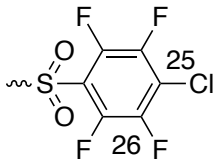
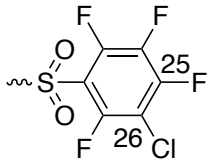
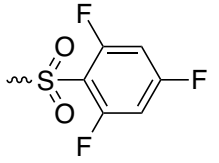
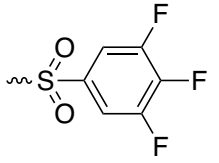
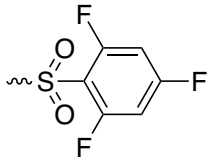
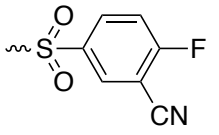
Compound	R_1	Y	IC_{50} µM
S3I-H206		CH	1.12
S3I-H172		N	0.98
S3I-H158		CH	2
S3I-H169		N	1.79
S3I-H177		N	4-5
S3I-224		N	1.08

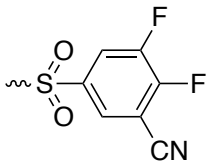
Compound	R_1	Y	IC_{50} µM
S3I-200		N	0.64
S3I-201		N	1.09

With the discovery that the fluorine atom at C25 is essential for activity at the DBD, it became crucial to determine whether the pentafluorophenyl was the optimal substructure to provide high potency, stability, and selectivity (**Figure 8**). Various substitution patterns of fluorine atoms were used to probe the effect of the R_4 region on the inhibition activity of the compounds (**Table 4**). Replacing the fluorine atom at C25 with a chlorine atom (**S31-H186**) led to no significant level of STAT3 inhibition. Replacement of the fluorine atom at C26 with a chlorine atom (**S3I-194**) led to inhibition of STAT3, lending support to the proposed mechanism of action of inhibition. Compounds containing three fluorine atoms (**S31-H007, S31-H101, S31-H101**) showed little to no activity. The use of the difluorocyano motif for this region (**S31-H203**) proved to be exciting due to a notable increase in potency of analogues containing this structure. This can be attributed to greater reactivity of the substrate due to the electron withdrawing effect of the nitrile. It was necessary to begin to examine ways to tune the reactivity of the system to S_NAr, so as to have desirable metabolic stability, and also pharmacokinetics.

Table 4. Select SAR of R_4 along with examples containing other scaffolds.



Compound	R2	X	R4	IC_{50} μM
S3I-H186	Pyrazine	(*R*)-Azetidine		>100
S3I-H194	Pyrazine	(*R*)-Azetidine		0.46
S3I-H080	Ph	(*R*)-Proline		>10.0
S3I-H007	Ph	CH_2		>30
S3I-H101	Ph	CH_2		>30
S3I-H113	Ph	(*R*)-Azetidine		21.9

Compound	R2	X	R4	IC_{50} μM
S3I-H203	Pyrazine	(*R*)-Azetidine		0.28

The first step in this work was the large-scale production of difluorocyanosulfonyl chloride for the use of the group in extensive SAR analysis. This allowed the group to produce numerous compounds containing this functionality. As these compounds were studied, it became apparent that there were problems with plasma stability. The work subsequently shifted focus to tune the S_NAr reactivity of the compounds so that they are still capable of reacting with a cysteine residue in the DBD of STAT3, but are not so reactive that they react indiscriminately with every nucleophile they may encounter. Significant effort was made to explore other aryl sulfonamides that would provide the desired reactivity. In this work a total of seven sulfonyl chlorides were made to explore reactivity in the DNA-binding domain of the STAT3 protein (**Figure 11**).

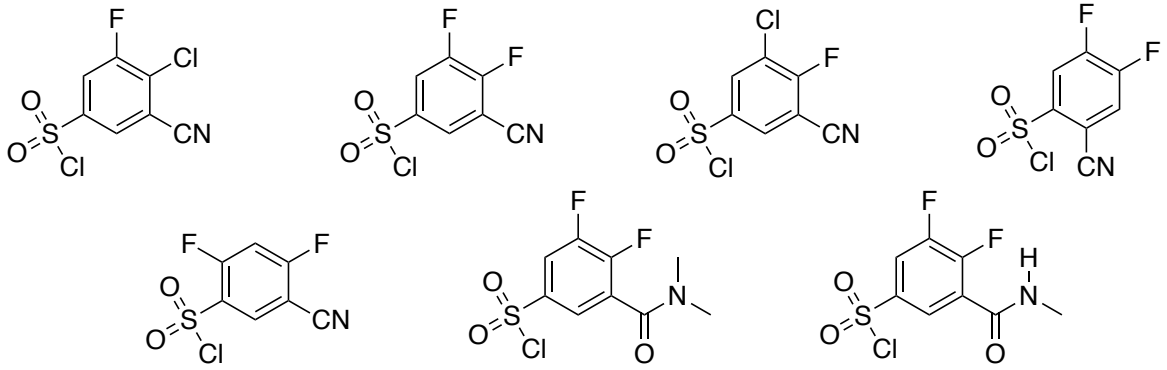


Figure 11. Aryl sulfonyl chlorides for varying R_4.

The inhibitors synthesized for this thesis were targeted as data was gathered that guided our design of promising new inhibitors. To study the SAR of inhibitors containing the difluorocyano motif, it was determined that targets **S3I-H240** and **S3I-H241** should be made (**Figure 12**). These inhibitors explored the pairing of simple aryl groups in R_1 with the difluorocyanobenzene motif with the goal of improving cell permeability. It was hypothesized that the inhibition activity of the compounds is largely from an S_NAr reaction with a cysteine residue in the DBD of STAT3. In order to support this hypothesis the fluorine atom at C25 was replaced with a chlorine atom to create **S3I-H254**. The next inhibitor was made by replacing the fluorine atom at C26 with a chlorine atom to create **S3I-H261**. These subsequent replacements of the fluorine atoms with chlorine atoms were made to support the mechanism of action of related compounds. Plasma stability data gathered by the Tius Group at this time indicated that the difluorocyanobenzene motif was too reactive to be viable as a drug. Subsequently, inhibitors **S3I-H265**, **S3I-H270**, **S3I-H275**, **S3I-H289** were made to explore the SAR of R_4 (**Figure 12**). These inhibitors were made in an attempt to tune the reactivity of the system to S_NAr. It was hypothesized that these inhibitors would react more selectively with the cysteine in the DBD of STAT3 and limit the reaction with extraneous nucleophiles in plasma, such as glutathione. It became apparent that constitutional isomers of the difluorocyano motif would not provide the desired potency and pharmacokinetics required to produce a candidate for clinical trials. Subsequently, it was decided that inhibitors **S3I-H247** and **S3I-H287** should be made in order to determine the effects of alkyl substitution, if any, at C13 (**Figure 8**). To explore inhibitors that replaced the nitrile at C24 in R_4 with a *N,N*-dimethylamide, inhibitors **S3I-H296** and **S3I-H299** were made. The final inhibitor synthesized, **S3I-H308,** contained an *N*-methylamide at C26.

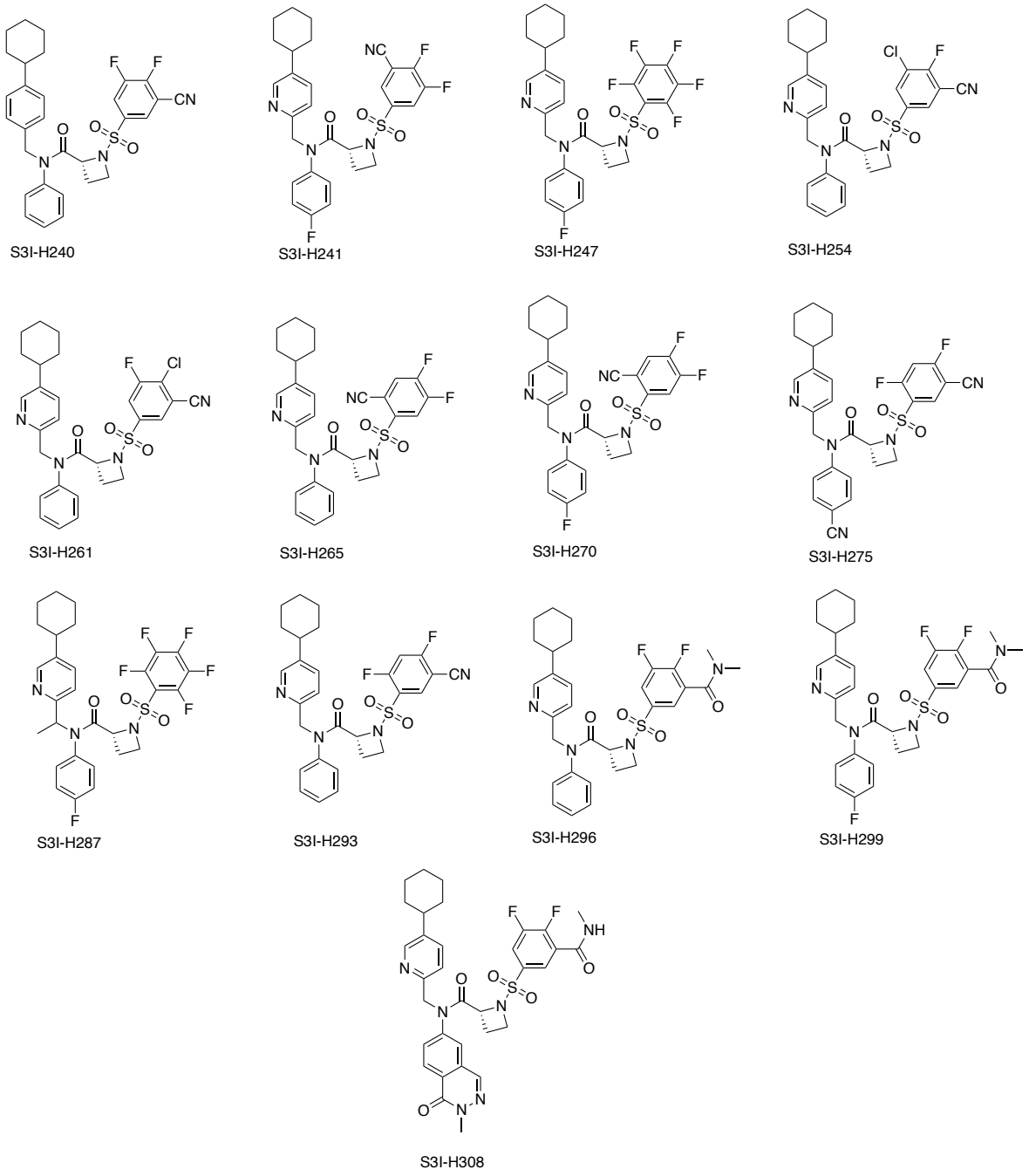
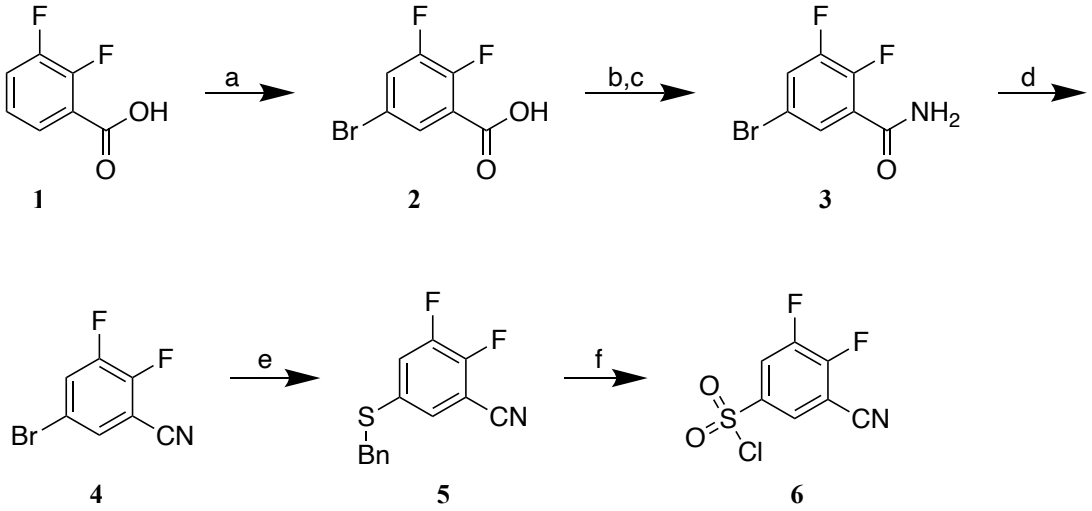


Figure 12. The synthetic targets of this thesis to interrogate the SAR of region R_4.

Chapter 2

Synthesis of Sulfonyl Chlorides and Targeted STAT3 Inhibitors

2.1 Synthesis of Large Quantities of 3-Cyano-4,5-Difluorobenzenesulfonyl Chloride



Scheme 1. Synthesis of 3-cyano-4,5-difluorobenzenesulfonyl chloride[a]

[a]Reagents and Conditions: a) H_2SO_4 (15.0 eq), NBS (1.05 eq), 60 ˚C, 3 h; b) oxalyl chloride (1.5 eq), DMF (cat.), DCM, 25 ˚C, 3 h; c) NH_4OH (excess), H_2O, 0 ˚C, 15 min; d) TFAA (1.1 eq), pyridine (2.0 eq), 1,4-dioxane, 0 to 25 ˚C, 4.5 h; e) $Pd_2(dba)_3$ (0.05 eq), Xantphos (0.1 eq), DIPEA (2.0 eq), BnSH (1.3 eq), 1,4-dioxane, reflux, 19 h, 65% over five steps; f) glacial acetic acid (4.5 eq), HPLC-grade H_2O (7.0 eq), trichloro-1,3,5-triazinane-2,4,6-trione (2.0 eq), MeCN, 0 to 25 ˚C, 1 h, 70%.

For the synthesis of 3-cyano-4,5-difluorobenzenesulfonyl chloride **Route A** was used and optimized (**Scheme 20**). The initial bromination step using NBS and sulfuric acid had two main problems:1, 2,3-dibromination, and, 2, lack of regioselectivity (**Scheme 1**). Quenching of the reaction after three hours was necessary in order to reduce dibromination. This reaction provided **2**, 6-bromo-2,3-difluorobenzoic acid and 2,3-dibromo-5,6-difluorobenzoic acid as an inseparable mixture. All subsequent substrates that were prepared according to these conditions have similar impurities. These impurities were removed at a later stage.

Carboxylic acid **2** was converted to the acid chloride and quenched with ammonium hydroxide to give amide **3**. The amide was next reacted with TFAA and pyridine to give **4**. It was found that increasing the scale required increased reaction times.

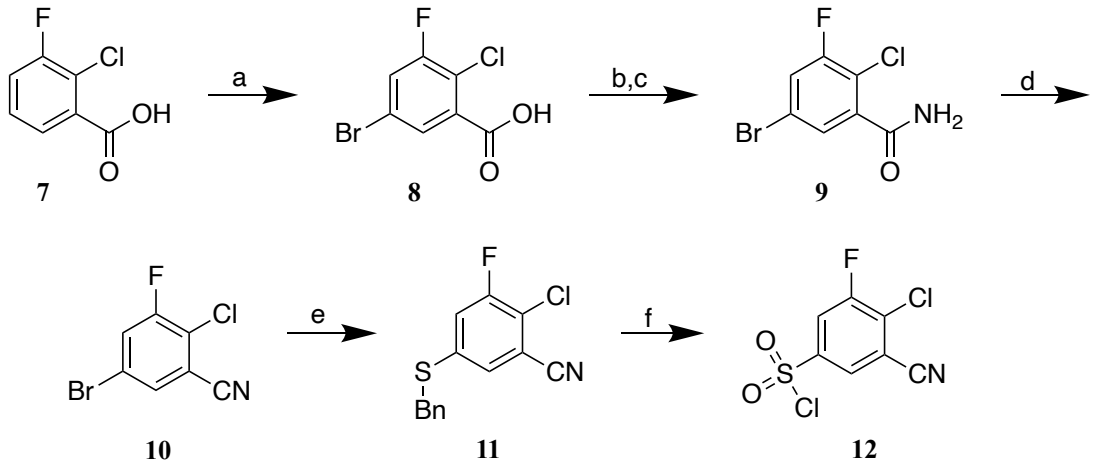
The Itoh cross coupling reaction was next utilized to introduce the thioether **5**. The reaction provided **5** in high yield, 65% over four steps. The reaction has two obstacles: 1, the mixture of isomers of the starting material and, 2, the recovery of the product was a challenging

separation by silica gel chromatography. Small scale procedures for this crucial purification required silica gel chromatography using 97:3 hexanes:EtOAc as the mobile phase, followed by trituration by hexanes. On large scale this was unsuitable for proper purification and a screening of eluents was done in order to improve chromatographic separation. Solubility issues were not an issue on small scale, but were a challenge on large scale. Prior equilibration of the silica gel with a different eluent (1:3 hexanes/toluene) was necessary in order to prevent the product from precipitating on silica gel.

Finally, the oxidation step could be performed to give **6** using glacial acetic acid, water, and 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione. Prior protocols for the isolation of **6** relied on silica gel chromatography. The isolation of **6** was improved upon by filtering the crude oil obtained from the reaction through a short silica plug to remove insoluble impurities. Then the desired product could be easily isolated by trituration with hexanes in adequate yield (70%). This procedure reduces the number of silica gel chromatographic separations to one for the entire sequence.

The conditions of purification of compounds **2**-**6** were used for all the sulfonyl chlorides that were synthesized using **Route A** (**Scheme 20**).

2.2 Synthesis of 3-cyano-4-chloro-5-fluorobenzenesulfonyl chloride



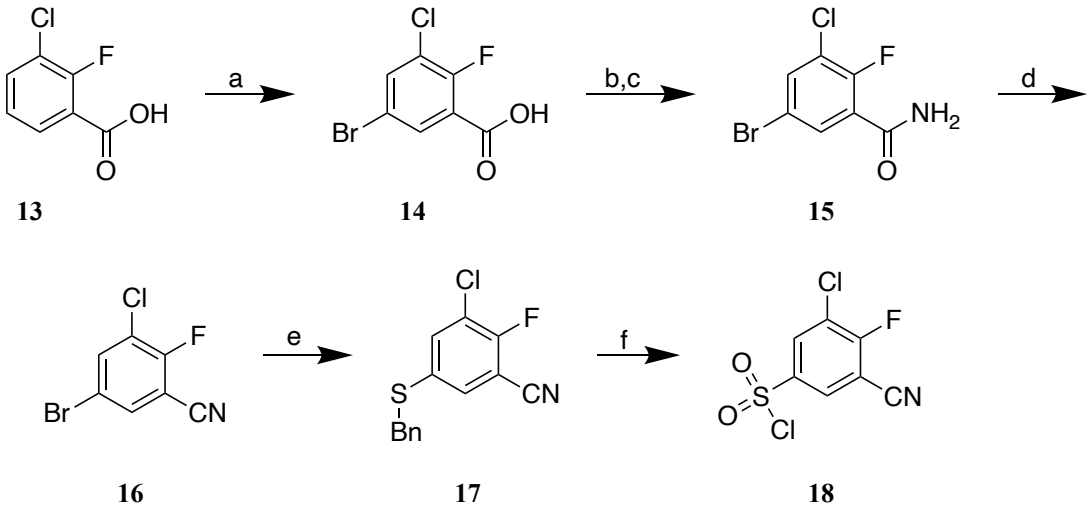
Scheme 2. Synthesis of 3-cyano-4-chloro-5-fluorobenzenesulfonyl chloride[a]

[a]Reagents and Conditions: a) H_2SO_4 (15.0 eq), NBS (1.5 eq), 60 °C, 3 h; b) oxalyl chloride (1.5 eq), DMF (cat.), DCM, 25 °C, 3 h, quantitative yield; c) NH_4OH (excess), H_2O, 0 °C, 15 min; d) TFAA (1.1 eq), pyridine (2.0 eq), 1,4-dioxane, 0 to 25 °C, 4.5 h; e) $Pd_2(dba)_3$ (0.05 eq), Xantphos (0.1 eq), DIPEA (1.9 eq), BnSH (1.05 eq), 1,4-dioxane, reflux, 19 h, 5% over five steps; f) glacial acetic acid (5.0 eq), HPLC-grade H_2O (8.0 eq), trichloro-1,3,5-triazinane-2,4,6-trione (2.2 eq), MeCN, 0 to 25 °C, 1 h, 40%.

The synthesis of 3-cyano-4-chloro-5-fluorobenzenesulfonyl chloride **12** was accomplished according to **Route A** (**Scheme 20**). With **7** the initial bromination step was considerably less selective than the bromination of **1** (**Scheme 2**). The product was impure, and the yield of this reaction was low. As judged by ^{19}F NMR, the purity of the material recovered was approximately 20 percent. There was a large amount of the 2,3-dibrominated species and 6-bromo-2-chloro-3-fluorobenzoic acid, a regioisomer of **8**. The impurities were separated at a later stage in the synthesis. The brominated carboxylic acid **8** was treated with oxalyl chloride and DMF in DCM followed by quenching with ammonium hydroxide, which gave **9**. The amide was dehydrated, and was converted to **10**. The Itoh reaction to introduce the thioether gave **11** in poor yield, 5% over five steps. At this stage the impurities generated from reaction a were separated from the desired compound. The oxidative step using 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione, glacial acetic acid and water gave **12** after filtration through a silica plug

and trituration with hexanes in low yield, 40%, but enough material was prepared to meet the goals of the project.

2.3 Synthesis of 3-chloro-5-cyano-4-fluorobenzenesulfonyl chloride

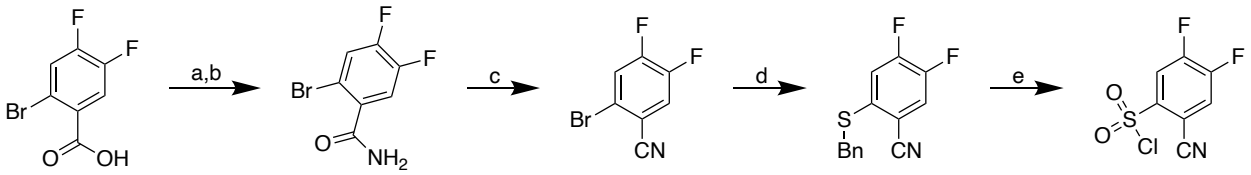


Scheme 3. Synthesis of 3-chloro-5-cyano-4-fluorobenzenesulfonyl chloride[a]

[a]Reagents and Conditions: a) H_2SO_4 (15.2 eq), NBS (1.05 eq), 60 °C, 3 h; b) oxalyl chloride (1.5 eq), DMF (cat.), DCM, 25 °C, 3 h; c) NH_4OH (excess), H_2O, 0 °C, 15 min; d) TFAA (1.1 eq), pyridine (2.0 eq), 1,4-dioxane, 0 to 25 °C, 4.5 h; e) $Pd_2(dba)_3$ (0.05 eq), Xantphos (0.1 eq), DIPEA (2.0 eq), BnSH (1.05 eq), 1,4-dioxane, reflux, 19 h, 24% oer five steps; f) glacial acetic acid (4.5 eq), HPLC-grade H_2O (7.2 eq), trichloro-1,3,5-triazinane-2,4,6-trione (2.0 eq), MeCN, 0 to 25 °C, 1 h, 43%.

3-Chloro-5-cyano-4-fluorobenzenesulfonyl chloride **18** was prepared according to **Route A** (**Scheme 20**). Bromination of **13** with NBS in sulfuric acid led to **14** (**Scheme 3**). The typical impurities were observed from these conditions. The impurities were separated at a later stage in the synthesis. Transformation of **14** to the acid chloride using oxalyl chloride and DMF in DCM followed by quenching with ammonium hydroxide gave **15**. Dehydration of **15** using TFAA and pyridine in dioxane gave **16**. Subjecting of **16** to Itoh reaction conditions provided the thioether in good yield, 24% over five steps. Using the standard oxidation procedure developed for **6** gave **18** in modest yield, 42%, but high purity. The decrease in yield was attributed to the formation of unidentified side products that were detected by TLC.

2.4 Synthesis of 2-cyano-4,5-difluorobenzenesulfonyl chloride



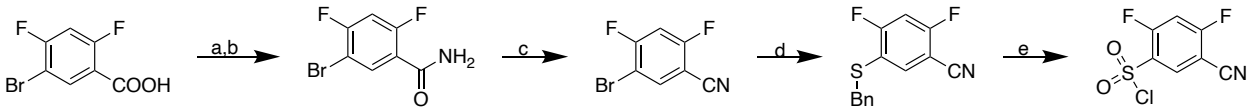
19 **20** **21** **22** **23**

Scheme 4. Synthesis of 2-cyano-4,5-difluorobenzenesulfonyl chloride[a]

[a]Reagents and Conditions: a) oxalyl chloride (1.4 eq), DMF (cat.), DCM, 25 °C, 3 h, quantitative yield; b) NH_4OH (excess), H_2O, 0 °C, 15 min, 91%; c) TFAA (1.2 eq), pyridine (2.0 eq), 1,4-dioxane, 0 to 25 °C, 4.5 h, 87%; d) $Pd_2(dba)_3$ (0.05 eq), Xantphos (0.1 eq), DIPEA (2.0 eq), BnSH (1.05 eq), 1,4-dioxane, reflux, 19 h, 55%; e) glacial acetic acid (4.5 eq), HPLC-grade H_2O (6.7 eq), trichloro-1,3,5-triazinane-2,4,6-trione (2.0 eq), MeCN, 0 to 25 °C, 1 h, 43 %.

2-Cyano-4,5-difluorobenzenesulfonyl chloride **23** was prepared according to **Route B** (**Scheme 20**). Conversion of **19** to the acid chloride using oxalyl chloride and DMF followed by quenching with ammonium hydroxide afforded **20** in excellent yield, 91% (**Scheme 4**). The dehydration of **20** to the nitrile using TFAA and pyridine in dioxane gave **21** in high yield, 87%. The conversion to the thioether using Itoh reaction conditions afforded **22** in modest yield, 55%. The modest yield was a consequence of the formation of several by products. These compounds were not isolated. The subsequent oxidation step using 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione, water, and acetic acid gave the desired product in modest yield (43%). Inefficient recovery of material after trituration was due to mechanical losses.

2.5 Synthesis of 5-cyano-2,4-difluorobenzenesulfonyl chloride



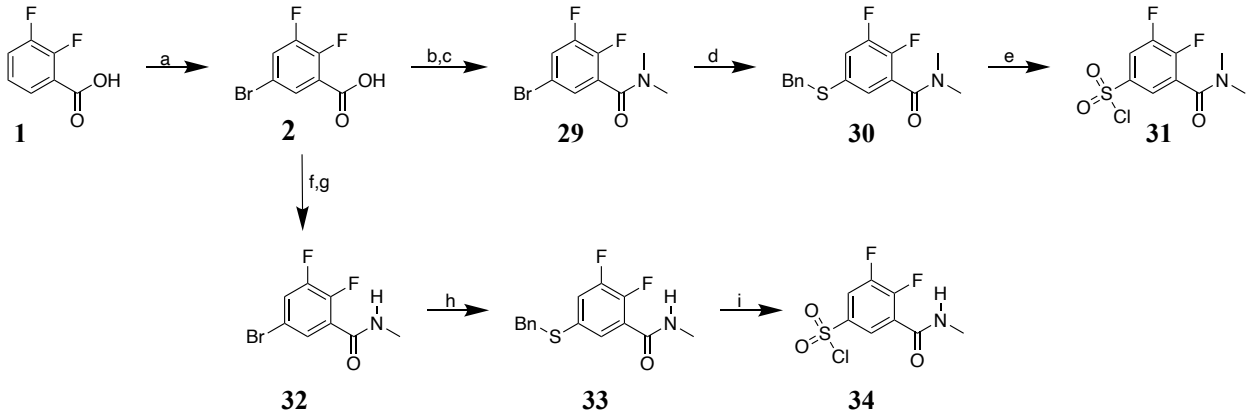
24 **25** **26** **27** **28**

Scheme 5. Synthesis of 5-cyano-2,4-difluorobenzenesulfonyl chloride[a]

[a]Reagents and Conditions: a) oxalyl chloride (1.4 eq), DMF (cat.), DCM, 25 °C, 3 h, quantitative yield; b) NH_4OH (excess), H_2O, 0 °C, 15 min, 93%; c) TFAA (1.3 eq), pyridine (2.0 eq), 1,4-dioxane, 0 to 25 °C, 4.5 h, 97%; d) $Pd_2(dba)_3$ (0.05 eq), Xantphos (0.1 eq), DIPEA (2.0 eq), BnSH (1.05 eq), 1,4-dioxane, reflux, 19 h, 73%; e) glacial acetic acid (4.5 eq), HPLC-grade H_2O (7.2 eq), trichloro-1,3,5-triazinane-2,4,6-trione (2.0 eq), MeCN, 0 to 25 °C, 1 h, 62%.

5-Cyano-2,4-difluorobenzenesulfonyl chloride **28** was prepared according to **Route B** (**Scheme 20**). Conversion of commercially available **24** to the acid chloride using oxalyl chloride and DMF in DCM took place in quantitative yield (**Scheme 5**). The acid chloride was then quenched with ammonium hydroxide to give **25** in excellent yield (93%). The conversion of the amide to **26** using TFAA and pyridine also proceeded in high yield (97%). The introduction of the thioether through the Itoh reaction provided **27** in adequate yield (73%). Oxidation of the sulfide to **26** using 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione, water and glacial acetic acid gave sulfonyl chloride **28** in 62% yield.

2.6 Synthesis of 3-(dimethylcarbamoyl)-4,5-difluorobenzenesulfonyl chloride and 3,4-difluoro-5-(methylcarbamoyl)benzenesulfonyl chloride



Scheme 6. Synthesis of 3-(dimethylcarbamoyl)-4,5-difluorobenzenesulfonyl chloride and 3,4-difluoro-5-(methylcarbamoyl)benzenesulfonyl chloride[a]

[a]Reagents and Conditions: a) H_2SO_4 (15.0 eq), NBS (1.1 eq), 60 °C, 3 h; b) oxalyl chloride (1.1 eq), DMF (cat.), DCM, 25 ˚C, 3 h; c) $NHMe_2$ (excess), H_2O, 0 ˚C, 15 min; d) $Pd_2(dba)_3$ (0.05 eq), Xantphos (0.1 eq), DIPEA (2.0 eq), BnSH (1.05 eq), 1,4-dioxane, reflux, 19 h, 50% over four steps; e) glacial acetic acid (4.0 eq), HPLC-grade H_2O (7.0 eq), 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione (2.0 eq), MeCN, 0 to 25 ˚C, 1 h, 45%; f) oxalyl chloride (1.1 eq), DMF (cat.), DCM, 0 to 25 ˚C, 3 h; g) NH_2Me (excess), H_2O, 0 ˚C, 15 min; h) $Pd_2(dba)_3$ (0.05 eq), Xantphos (0.1 eq), DIPEA (2.0 eq), BnSH (1.2 eq), 1,4-dioxane, reflux, 19 h, 21% over four steps; i) glacial acetic acid (4.2 eq), HPLC grade- H_2O (7.4 eq), 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione (2.0 eq), MeCN, 0 to 25 ˚C, 1 h, 28%.

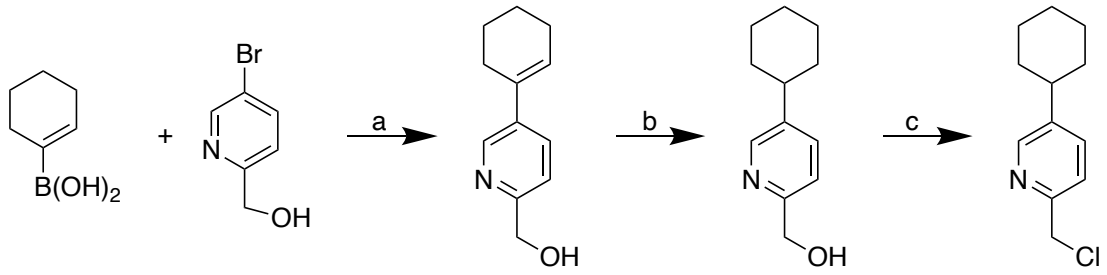
3-(Dimethylcarbamoyl)-4,5-difluorobenzenesulfonyl chloride **31** was accomplished according to **Route C** (**Scheme 20**). Commercially available **1** was brominated with NBS in sulfuric acid to **2** (**Scheme 6**). The product was contaminated by the 2,3-dibrominated species, and 6-bromo-2,3-difluorobenzoic acid. The impurities were removed at a later step in the synthesis.

The conversion of the carboxylic acid to the acid chloride was done using standard reaction conditions (**Scheme 6**). Conversion of the acid chloride to **29** was accomplished by addition of the acid chloride to dimethylamine in DCM. The resulting mass of the recovered product was lower than expected. This was attributed to an uncertainty in the measured amount of dimethylamine because the amine hydrochloride was wet. The reaction provided sufficient material and was not optimized further. The dimethyl amide was treated under Itoh reaction

conditions to provide **30** in 50% yield over four steps. The impurities were removed at this stage by silica gel chromatography. The oxidation from the thioether to the sulfonyl chloride **31** gave the desired compound in modest yield (45%).

3,4-Difluoro-5-(methylcarbamoyl)benzenesulfonyl chloride **34** was prepared according to **Route C** (**Scheme 20**). Conversion of **2** to the acid chloride using oxalyl chloride and DMF in DCM followed by addition of a solution of 30% methylamine provided **32** (**Scheme 6**). The Itoh reaction to produce the thioether provided **33** in modest yield, 21% over four steps. The impurities generated in reaction a were removed by silica gel chromatography at this stage. Finally, the oxidation to the sulfonyl chloride using the standard conditions developed for **6** gave **34** in modest yield (28%) but in high purity. A large amount of side product formed as judged by TLC. It was hypothesized that **34** had undergone hydrolysis or nucleophilic aromatic substitution. The reaction was not optimized.

2.7 Synthesis of Alkyl Chloride 39



35 **36** **37** **38** **39**

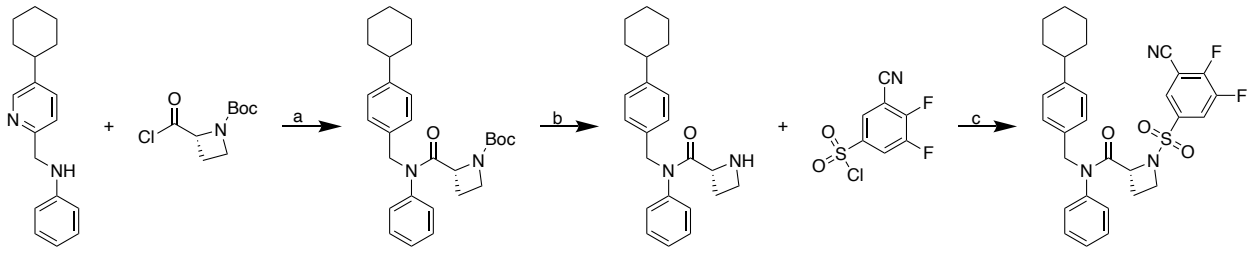
Scheme 7. Synthetic of **39**[a]

[a]Reagents and Conditions: Route A - a) SPhos (0.1 eq), $Pd(OAc)_2$ (0.05 eq), K_3PO_4 (2.0 eq), H_2O (2.0 eq), THF, 40 °C, 24 h, 95% yield; b) H_2, PtO_2 (10% by weight), 1:1 EtOAc:MeOH, rt, 24 h, 97% yield; c) d) $SOCl_2$ (1.2 eq), DCM, rt, 3 h, quantitative yield.

The gram scale synthesis of **39** began with a Suzuki cross-coupling reaction (**Scheme 7**). Alkene **37** was formed reproducibly in 90-95% yield. In a previously reported synthesis of **39** by the group this reaction had been low yielding, but was high yielding in my hands. Subsequent reduction of the olefin by Adams catalyst under a H_2 atmosphere gave **38** in 97% yield. It is necessary to monitor this reaction by ^1H NMR for completeness because **37** and **38** are not easily

distinguished by TLC. Conversion of **38** to **39** using thionyl chloride in DCM provided the desired product in quantitative yield. The alkyl chloride was either stored as the hydrochloride salt if the reaction was concentrated directly or as the free-base in toluene (0.5 M) at -20 °C after basic work up. This synthesis is reproducible at the multi-gram scale, which provided ample intermediate for the convergent syntheses of STAT3 inhibitors.

2.8 Synthesis of S3I-H240



67 **48** **68** **69** **6** **S3I-H240**

Scheme 8. Synthesis of **S3I-H240**[a]

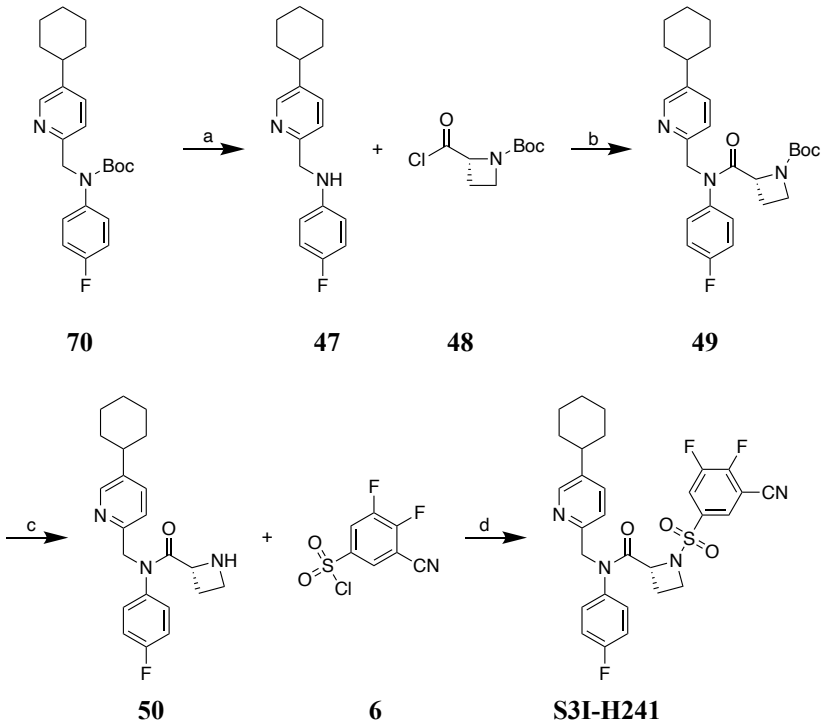
[a]Reagents and Conditions: a) MeMgBr (2.5 eq), **48** (2.0 eq), THF, 0 to 25 °C, 1 h, 25%; b) TFA (37.0 eq), DCM, rt, 1h; f) DIPEA (6.0 eq), **6** (1.3 eq), DCM, 0 to 25 °C, 2.5 h, 41% over two steps.

The first inhibitor was made from **67** (**Scheme 8**). Addition of MeMgBr (1.4 M) **67** in THF deprotonated the aniline nitrogen atom. Addition of **48** to deprotonated **67** afforded **68** in poor yield (25%). This reaction was problematic in several substrates for reasons that were unclear until it was discovered that there were concentrations of peroxides in excess of 100 ppm in THF from the SPS system that had been used as the reaction solvent. There was also γ-butyrolactone in the THF as a result of the peroxide decomposition. After the discovery of the contaminants in the SPS system the amount of Grignard reagent used was lowered from 2.5 equivalents to 1.3 equivalents. Following poor results with 1.3 equivalents of base. The amount of Grignard reagent used was increased to 1.6, 1.8, and 2.5 equivalents sequentially. These results suggest that the excess of Grignard reagent that had been used earlier on by the other group members was not because of the contamination of the THF. The reaction was extremely

dependent on the excess of base. The optimal amount of MeMgBr for reactions using similar conditions is 2.5 equivalents. It is unclear why such a large excess is needed.

Deprotection of anilide **68** with excess TFA in DCM followed by concentration and direct use in the the subsequent reaction deviates from procedures used by some group members. It had been previously reported that a basic work up followed by silica gel chromatography was necessary after the deprotection, however **69** and amines like it were used crude in the subsequent reaction. To crude **69** excess DIPEA was added. After waiting fifteen minutes, **6** was added as a solution in DCM. Satisfyingly, this procedure gave the desired target, **S3I-H240**, in 41% yield over two steps. This alteration eliminates both a work up and purification, thus improving scalability.

2.9 Synthesis of S3I-H241

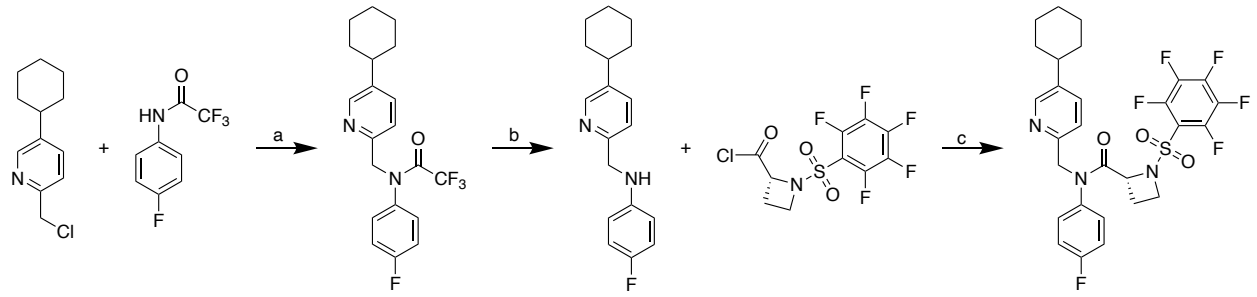


Scheme 9. Synthesis of inhibitor **S3I-H241**[a]

[a]Reagents and Conditions: a) TFA (37.0 eq), DCM, rt, 1 h, 85%; b) MeMgBr (2.5 eq), **48** (2.0 eq), THF, 0 to 25 °C, 1 h, 33%; c) TFA (37.0 eq), DCM, rt, 1h; d) DIPEA (6.0 eq), **6** (1.3 eq), DCM, 0 to 25 °C, 2.5 h, 31% over two steps.

The synthesis of **S3I-H241** began from protected carbamate, **70** (**Scheme 9**). The carbamate was deprotected using excess TFA in DCM to give aniline **47** in 85% yield. The aniline was then deprotonated using MeMgBr (1.4 M). After waiting twenty minutes to allow the substrate to be fully deprotonated, freshly prepared **48** was added as a solution in THF. This reaction provided anilide **49** in 33% yield. The reaction's poor yield can be attributed to high concentrations of contaminants in the reaction solvent as described for **Scheme 8**. With the desired carbamate in hand the synthesis was carried forward. Compound **49** was deprotected using excess TFA in DCM. After one hour the reaction mixture was concentrated. The solid crude product was then used directly in reaction d (**Scheme 9**). Excess DIPEA was added to **50** in DCM, and after fifteen minutes **6** was added to the reaction mixture leading to the target, **S3I-H241**, in 31% yield over two steps.

2.10 Synthesis of S3I-H247



Scheme 10. Synthesis of **S3I-H247**[a]

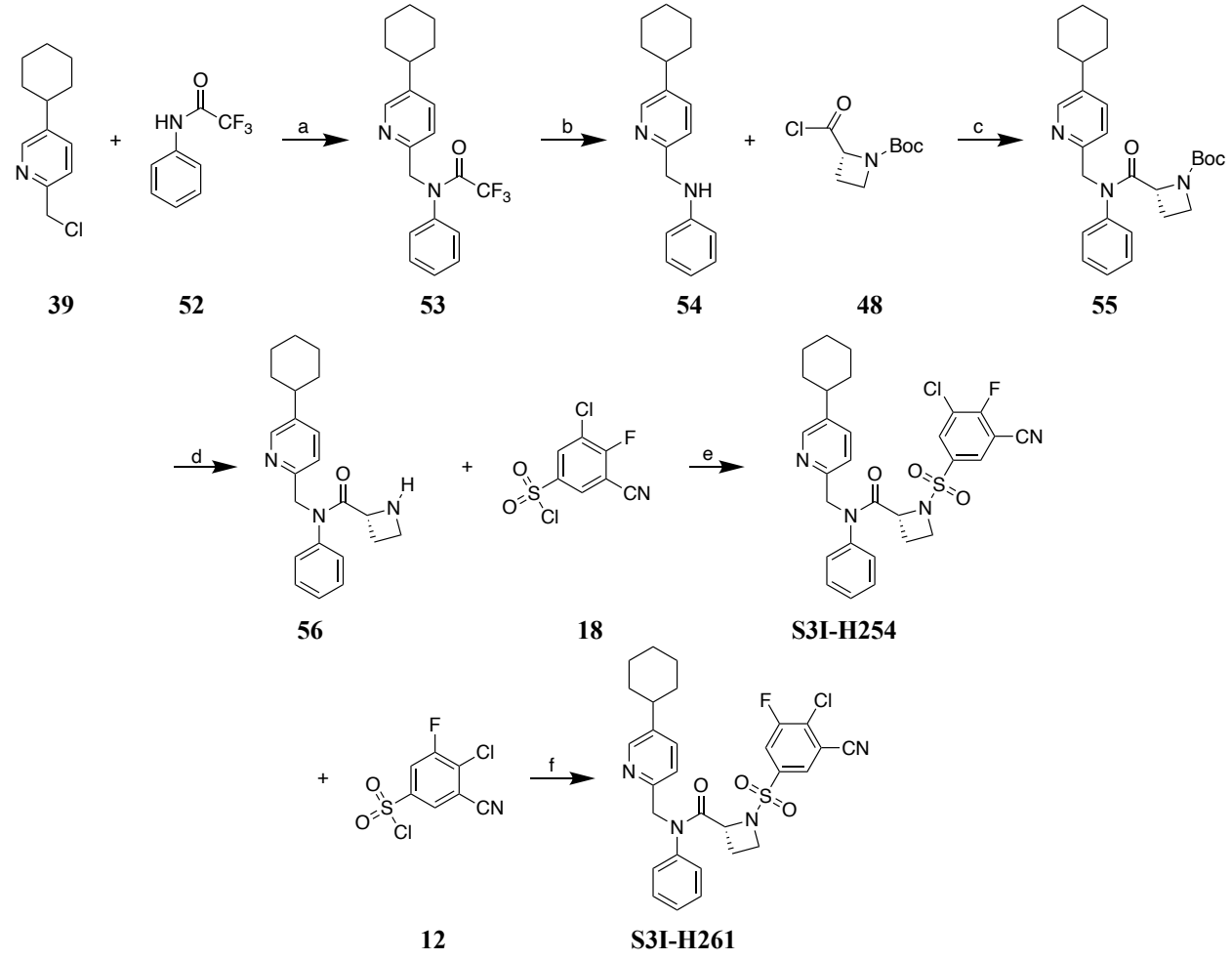
[a]Reagents and Conditions: a) **39** (2.0 eq), NaI (0.2 eq), K_2CO_3 (2.0 eq), MeCN, 60 °C, 16 h; b) K_2CO_3 (2.0 eq), 1:1 THF:MeOH, rt, 24 h, 52% over two steps; c) MeMgBr (2.6 eq), **66** (2.0 eq), THF, 0 to 25 °C, 1 h, 20%.

The synthesis of **S3I-H247** followed **Route B** (**Scheme 22**). The S_N2 reaction between chloride **39** and anilides such as **45** was always catalyzed by the addition of 0.2 equivalents of sodium iodide (**Scheme 10**). This reaction did not go to completion; unreacted starting material was detected via TLC. Filtration through a pad of silica gel afforded a light green oil. The crude product was used directly in the next reaction in which the trifluoroacetamide **46** was deprotected

with potassium carbonate in THF:MeOH (1:1). This gave **47** in 52% yield over two steps. When this procedure was used, purification by silica gel chromatography was not necessary.

Amine **46** was deprotonated with MeMgBr (1.4 M). After addition of **66**, followed by silica gel column chromatography, the yield of **S3I-H247** was 20%. It was unclear at this point what was causing the low yield in the last step of this synthesis. This reaction was not optimized, as it provided a significant quantity of the desired target **S3I-H247**.

2.11 Synthesis of S3I-H254 and S3I-H261



Scheme 11. The synthesis of **S3I-H254** and **S3I-H261**[a]

[a]Reagents and Conditions: a) **39** (2.0 eq), Cs_2CO_3 (4.5 eq), NaI (0.2 eq), MeCN, 65 ˚C, 16 h; b) K_2CO_3 (2.0 eq), 1:1 THF:MeOH, 3 h, 50% over two steps; c) MeMgBr (2.5 eq), **48** (2.0 eq), THF, 0 to 25 ˚C, 1 h, 43%; d) TFA (37.0 eq), DCM, rt, 1h; e) DIPEA (6.0 eq), **18** (1.3 eq), DCM, 0 to 25 ˚C, 2.5 h, 30% yield over two steps; f) DIPEA (6.0 eq), DCM, 0 to 25 ˚C, 2.5 h, 45% over two steps.

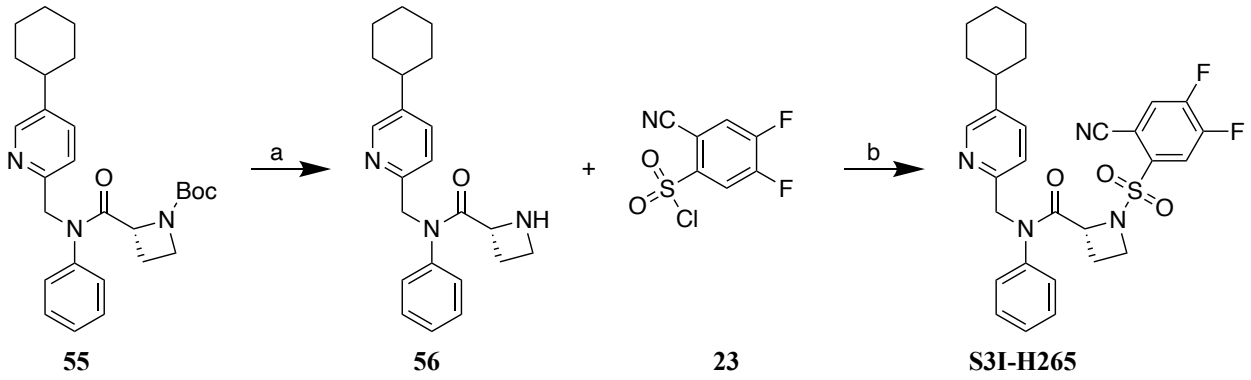
The synthesis of **S3I-H254** followed **Route A** (**Scheme 22**). It was hypothesized that the alkyl chloride could be concentrated directly after preparation and stored as a bench stable hydrochloride salt. To test whether the hydrochloride salt could be used in place of the free base it was used in the next reaction. This would be an improvement as it was a solid. The S_N2 reaction between **39** as the hydrochloride salt and **52** led to **53** (**Scheme 11**). In order to effectively deprotonate the hydrochloride salt of **39**, Cs_2CO_3 was used as the base in large excess (4.5 equivalents). The resulting trifluoroacetamide **53** was filtered through silica gel and then used in the subsequent reaction. Trifluoroacetamide **53** was deprotected with base, and purified via silica gel chromatography. This provided **54** in 50% yield over two steps.

Aniline **54** was deprotonated with MeMgBr (1.4 M). Freshly prepared **48** was added to **54** as a solution in THF (**Scheme 11**). This reaction gave anilide **55** in 43% yield. Carbamate **55** was deprotected using excess TFA in DCM. The mixture was allowed to stir for an hour, and concentrated for use in the next reaction. Hünig's base was added to crude **56**, and after 15 minutes **18** was added as a solution in DCM. This gave **S3I-H254** in 30% yield over two steps.

The synthesis of **S3I-H261** began with the S_N2 reaction of the hydrochloride salt of **39** and **52** (**Scheme 11**). This reaction gave **53** as a crude oil. The crude oil was filtered through silica gel and used directly in the subsequent reaction. Crude **53** was deprotected with base to give **54** in 53% yield over two steps. Satisfyingly, this yield is congruent with previous results from the synthesis of **S3I-H254** (**Scheme 11**).

The synthesis continued with the transformation of **54** to anilide **55** (**Scheme 11**). It was at this time that the contamination in the THF was discovered. The product, **54**, was formed in a disappointing 19% yield. The resulting anilide was divided evenly into two portions and one was saved for another target. Deprotection of **55** by exposure to excess TFA in DCM gave crude **56** after concentration. Addition of excess Hünig's base to crude **56**, followed by **12** as a solution in DCM gave **S3I-H261** in 45% yield over two steps.

2.13 Synthesis of S3I-H265

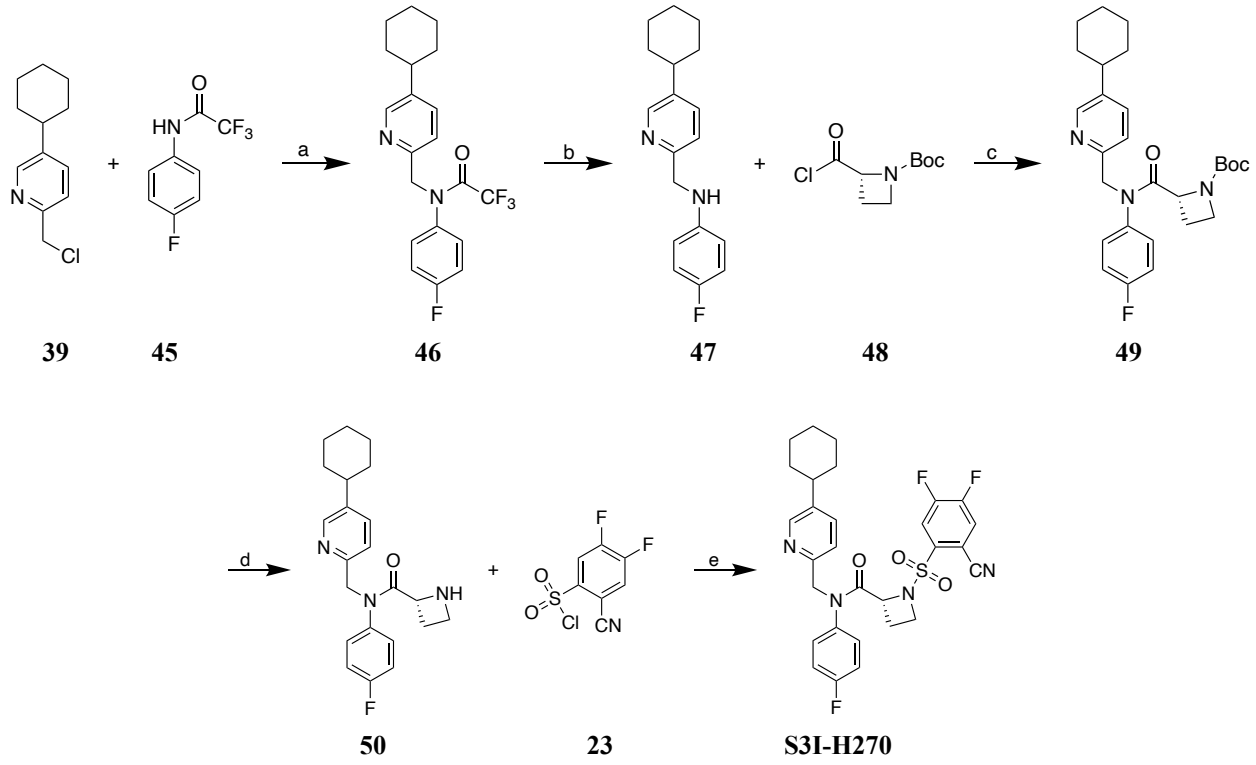


Scheme 12. Synthesis of **S3I-H265**[a]

[a]Reagents and Conditions: a) TFA (37.0 eq), DCM, rt, 1h; b) DIPEA (6.0 eq), **23** (1.3 eq), DCM, 0 to 25 °C, 2.5 h, 36% over two steps.

S3I-H265 was identified as a target close to the time that **S3I-H261** was. The utility of a convergent route was demonstrated by starting with a common intermediate **55** (**Scheme 12**). The intermediate from synthesis of **S3I-H261** that had been saved was deprotected using excess TFA in DCM. After one hour the solvent was removed under reduced pressure and the resulting crude oil was used directly in the next reaction. Excess Hünig's base was added to crude **56**, and after fifteen minutes **23** was added to the reaction mixture to give **S3I-H265** in 36% yield over two steps.

2.14 Synthesis of S3I-H270



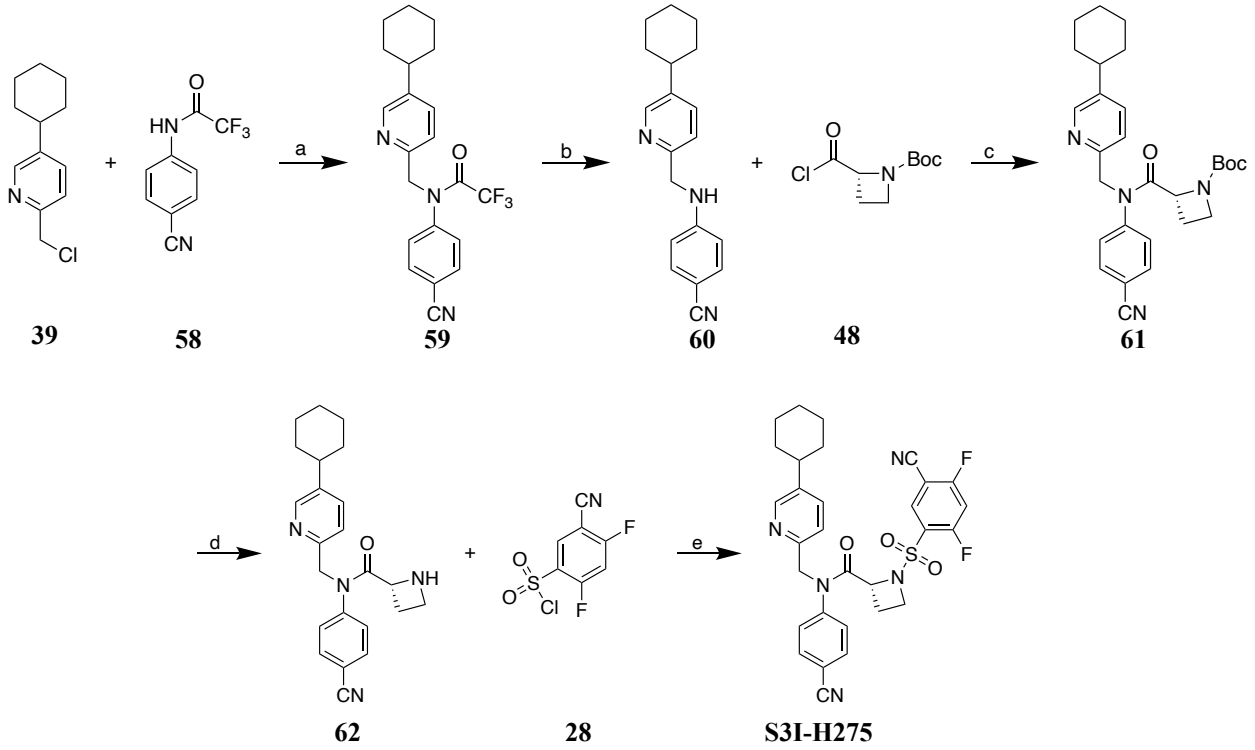
Scheme 13. Synthesis of **S3I-H270**[a]

[a]Reagents and Conditions: a) **39** (2.0 eq), Cs_2CO_3 (4.5 eq), NaI (0.2 eq), MeCN, 60 ˚C, 16 h; b) K_2CO_3 (4.5 eq), 1:1 THF:MeOH, 3 h, 65% over two steps; c) MeMgBr (1.3 eq), **48** (2.0 eq), THF, 0 to 25 ˚C, 1 h, 30%; d) TFA (37.0 eq), DCM, rt, 1h; e) DIPEA (6.0 eq), **6** (1.3 eq), DCM, 0 to 25 ˚C, 2.5 h, 38% over two steps.

The synthesis of **S3I-H270** began with the S_N2 reaction of the hydrochloride salt of **39** and **45** (**Scheme 13**). Crude **46** was filtered through silica gel and then used directly in reaction b. Deprotection under basic conditions gave the product **47** in 65% yield over two steps. The high yields of the two steps, coupling and removal of the trifluoroacetyl group, as well as needing only one purification step, led to high yields of **47** and of all other related anilines.

The deprotonation of the nitrogen atom of aniline **47** was performed utilizing only a slight excess of MeMgBr (**Scheme 13**). This reaction gave anilide **49** in 30% yield. Following purification **49** was deprotected and concentrated, giving crude **50**. Addition of Hünig's base to crude **50** followed by **23** as a solution in DCM gave **S3I-H270** in 38% yield over two steps.

2.15 Synthesis of S3I-H275



Scheme 14. Synthesis of **S3I-H275**[a]

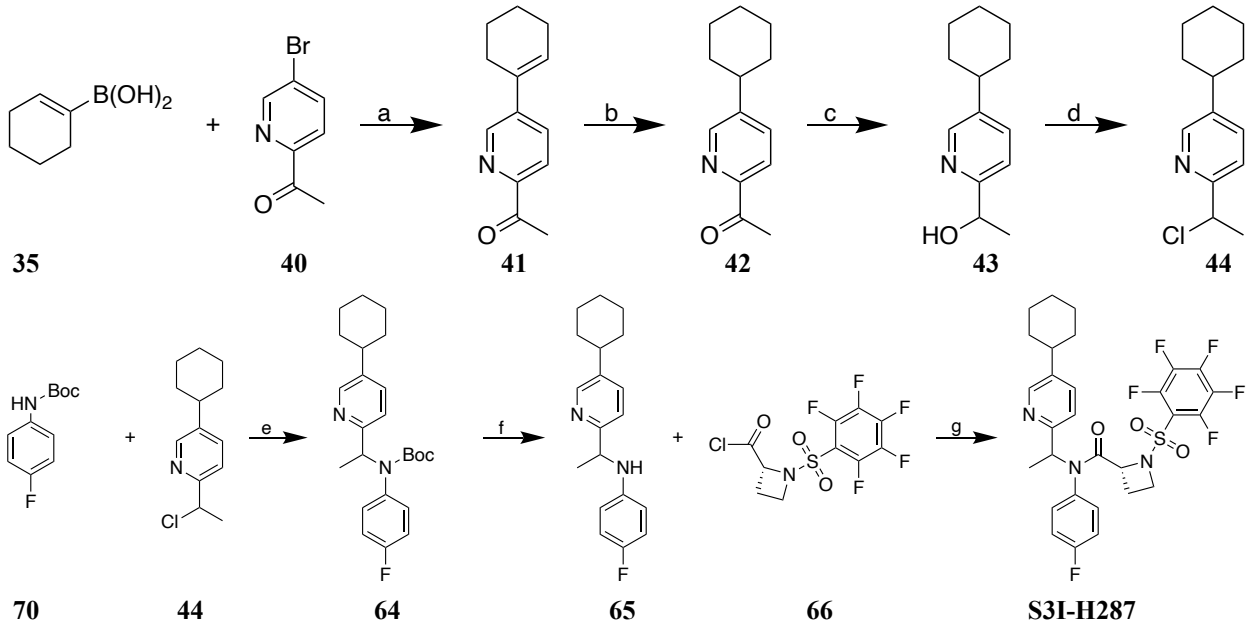
[a]Reagents and Conditions: a) **39** (1.5 eq), K_2CO_3 (2.0 eq), NaI (0.2 eq), MeCN, 60 ˚C, 16 h; b) K_2CO_3 (2.0 eq), 1:1 THF:MeOH, 3 h, 35% over two steps; c) MeMgBr (1.3 eq), **48** (2.0 eq), THF, 0 to 25 ˚C, 1 h, 9%; d) TFA (37.0 eq), DCM, rt, 1h; e) DIPEA (6.0 eq), **28** (1.3 eq), DCM, 0 to 25 ˚C, 2.5 h, 43% over two steps.

The synthesis of **S3I-H275** began with the S_N2 reaction of **39** and **58** to give crude trifluoroacetamide **59** (**Scheme 14**). One and a half equivalents of **39** were used in order to determine if the 2.0 equivalents of **39** used in the previous syntheses were necessary. Two equivalents of chloride **39** were determined to be the optimal conditions. The crude product was filtered through silica gel and used directly in the next reaction. Aniline **60** was obtained in 35% yield over two steps. Using these conditions this substrate led to a slightly lower yield than was seen in similar reactions, but the reaction provided ample material to carry the synthesis forward.

Aniline **60** was deprotonated with 1.3 equivalents of MeMgBr (1.4 M). After 15 minutes, **48** was added to the reaction mixture (**Scheme 14**). The anilide was obtained in 9% yield. Later,

the experiment was repeated with 2.5 equivalents of base and the yield was improved to 58%. Protected **61** was exposed to excess TFA in DCM and concentrated. To crude **62** was added excess Hünig's base followed by sulfonyl chloride **28** to provide **S3I-H275** in 43% yield over two steps.

2.16 Synthesis of S3I-H287



Scheme 15. Synthesis of **S3I-H287**[a]

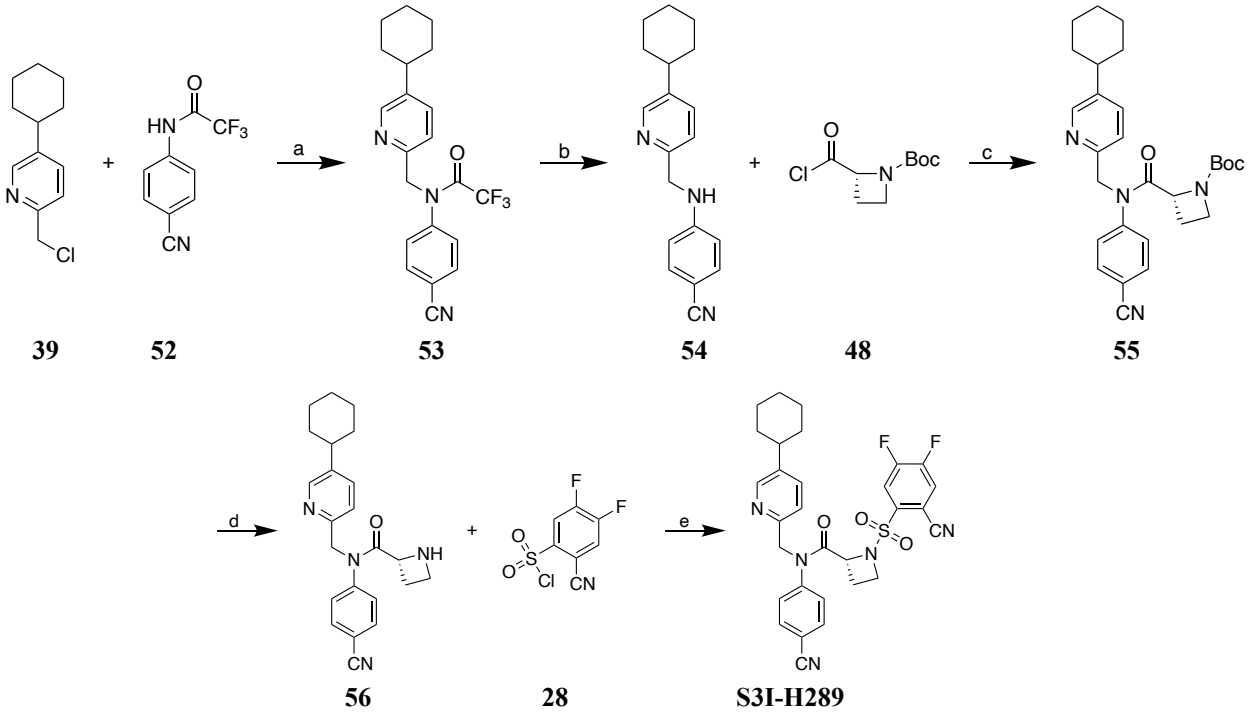
[a]Reagents and Conditions: a) SPhos (0.1 eq), $Pd(OAc)_2$ (0.05 eq), K_3PO_4 (2.0 eq), H_2O (2.0 eq), THF, 40 ˚C, 24 h, 58%; b) H_2, PtO_2 (10% by weight), 1:1 EtOAc:MeOH, rt, 24 h, quantitative; c) $NaBH_4$ (5.0 eq), MeOH, 0 ˚C, 3 h, 93%; d) $SOCl_2$ (1.2 eq), DCM, rt, 3 h, quantitative yield; e) **44** (2.0 eq) NaI (0.2 eq), Cs_2CO_3 (4.5 eq), MeCN, 60 ˚C, 16 h; f) TFA (37.0 eq), DCM, rt, 24 h, 57% over two steps; g) MeMgBr (1.3 eq), **66** (2.0 eq),THF, 0 to 25 ˚C, 1 h, 6%.

The synthesis of **S3I-H287** required chloride **44** (**Scheme 21**). Using Suzuki reaction conditions, aryl bromide **40** was coupled to boronic acid **35** in moderate yield (58%; **Scheme 15**). The reaction to produce **41** was performed only once. Reduction of **41** using Adams catalyst provided **42** in quantitative yield. Reduction of the ketone **42** using sodium borohydride in methanol provided alcohol **43** in excellent yield (93%). Exposure of **43** to thionyl chloride

afforded alkyl chloride **44** in quantitative yield. With the desired alkyl chloride in hand, synthetic **Route B** was used to prepare **S3I-H287** (**Scheme 22**). The S_N2 reaction of **44** and **70** gave **64**. The carbamate **64** was used directly in the next reaction. Deprotection of **64** using TFA gave **65** in 57% yield over two steps.

The reaction of **65** with MeMgBr followed by addition of **66** gave **S3I-H287** in low yield, 6% (**Scheme 16**). This reaction was done on a small scale, less than 30 milligrams, and therefore the mechanical losses were significant. Numerous unknown side products were formed in this reaction. There was an impurity that was detected by LCMS that proved to be inseparable by silica gel chromatography as well as preparative TLC. To recover enough pure **S3I-H287** for biological testing the crude compound was purified by HPLC. Product **S3I-H287** was submitted as a mixture of diastereomers, and it is unclear what effect this has on the biological activity of the target, if any.

2.17 Synthesis of S3I-H289

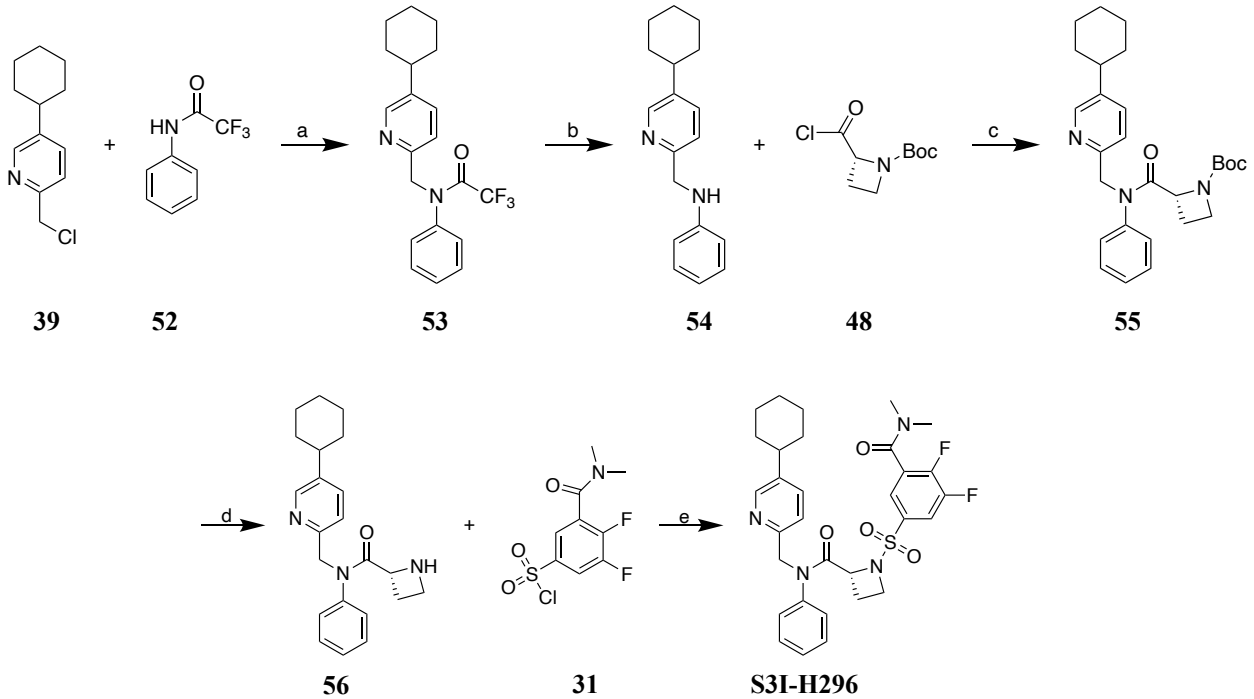


Scheme 16. Synthesis of **S3I-H289**[a]

[a]Reagents and Conditions: a) **39** (1.3 eq), K_2CO_3 (2.5 eq), NaI (0.2 eq), MeCN, 65 °C, 16 h; b) K_2CO_3 (2.5 eq), 1:1 THF:MeOH, 3 h, 38% over two steps; c) MeMgBr (1.3 eq), **48** (2.0 eq), THF, 0 to 25 °C, 1 h, 22%; d) TFA (37.0 eq), DCM, rt, 1h, quantitative yield; e) DIPEA (6.0 eq), **28** (1.3 eq), DCM, 0 to 25 °C, 2.5 h, 41% yield over two steps.

The synthesis of target **S3I-H289** began with the S_N2 reaction of **52** and **39** (**Scheme 16**). In this reaction the equivalents of **39** were decreased (1.3 equivalents). After filtration through silica gel the crude product was used in the next reaction. The aniline **54** was procured in 38% yield over two steps. This is a lower yield than when 2.0 equivalents of **39** were used in the reaction to make **54** for **S3I-H254** (**Scheme 11**). The next reaction provided **55** in low yield (22%). This reaction confirmed that it was necessary to change the reaction conditions in order to improve the yield. Accordingly, the equivalents of base were increased in a repetition of the reaction. This provided anilide **55** in 58% yield. The anilide **55** was deprotected using TFA in DCM. The reaction mixture was concentrated and the crude oil was used directly in the next reaction. Excess Hünig's base was added to crude **56** followed by sulfonyl chloride **28**. This gave **S3I-H289** in 41% yield over two steps.

2.18 Synthesis of S3I-H296



39 **52** **53** **54** **48** **55**

56 **31** **S3I-H296**

Scheme 17. Synthesis of **S3I-H296**[a]

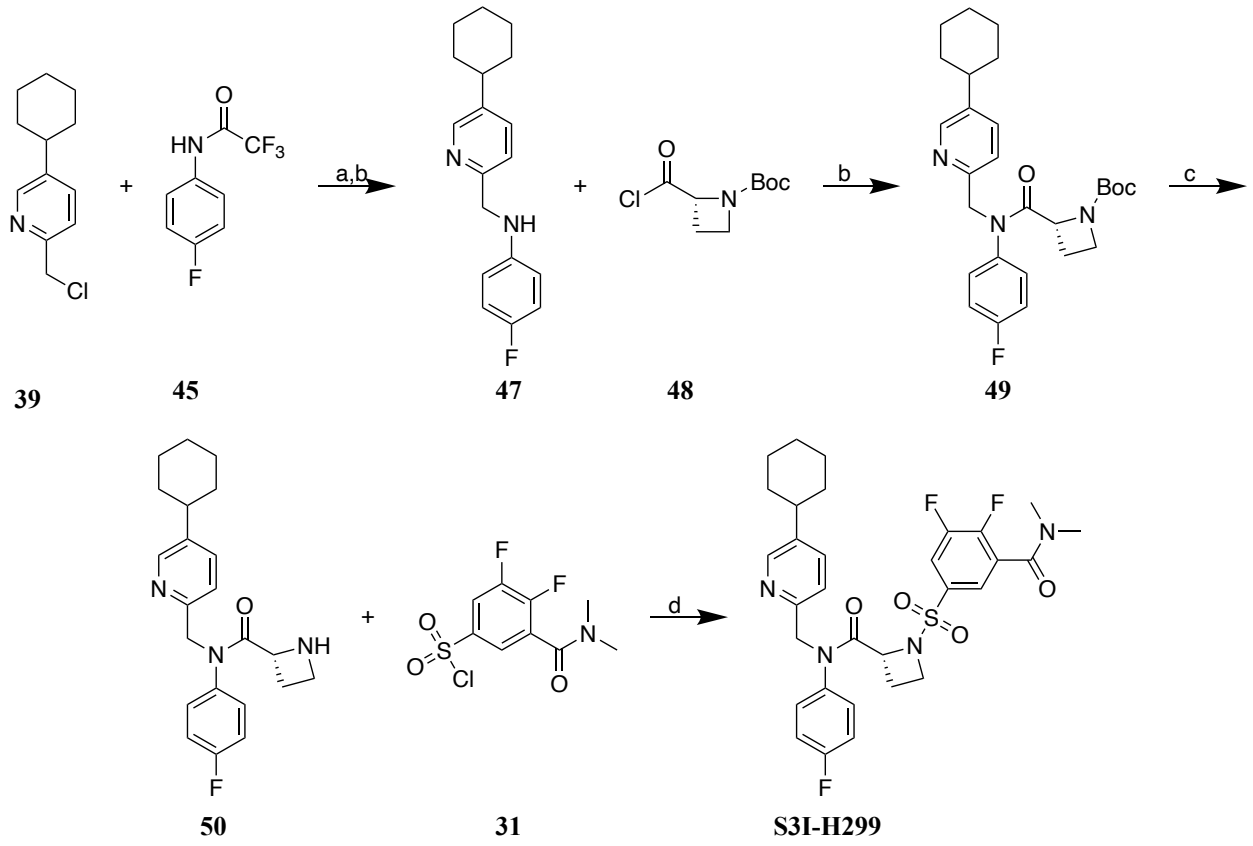
[a]Reagents and Conditions: a) **39** (2.0 eq), Cs_2CO_3 (4.5 eq), NaI (0.2 eq), MeCN, 65 ˚C, 16 h; b) K_2CO_3 (2.5 eq), 1:1 THF:MeOH, 3 h, 47% over two steps; c) MeMgBr (1.6 eq), **48** (2.0 eq), THF, 0 to 25 ˚C, 1 h, 18%; d) TFA (37 eq), DCM, rt, 1h; e) DIPEA (6 eq), **31** (1.3 eq), DCM, 0 to 25 ˚C, 2.5 h, 30% over two steps.

The synthesis of **S3I-H296** followed **Route A** (**Scheme 22**). The S_N2 reaction of the starting materials, **39** and **52**, led to **53** (**Scheme 17**). The crude solid was used directly in the next reaction. Crude trifluoroacetamide **53** was deprotected with potassium carbonate in THF:MeOH (1:1), to give **54** in 47% yield over two steps. This is similar to the yield of **54** when it was prepared in the synthesis of **S3I-H254** (**Scheme 11**). The use of two equivalents of alkyl chloride and an excess (4.5 equivalents) of cesium carbonate as the base is optimal for this reaction.

Deprotonation of **54** with 1.6 equivalents of MeMgBr followed by addition of **48** gave anilide **55** in 18% yield. The reaction provided significant amounts of **55** and was not further optimized.

Anilide **54** was deprotected with excess TFA in DCM and concentrated. The resulting crude oil was used directly in the following reaction. To crude **56** was added excess DIPEA followed by **31**. This gave **S3I-H296** in 30% yield over two steps.

2.19 Synthesis of S3I-H299



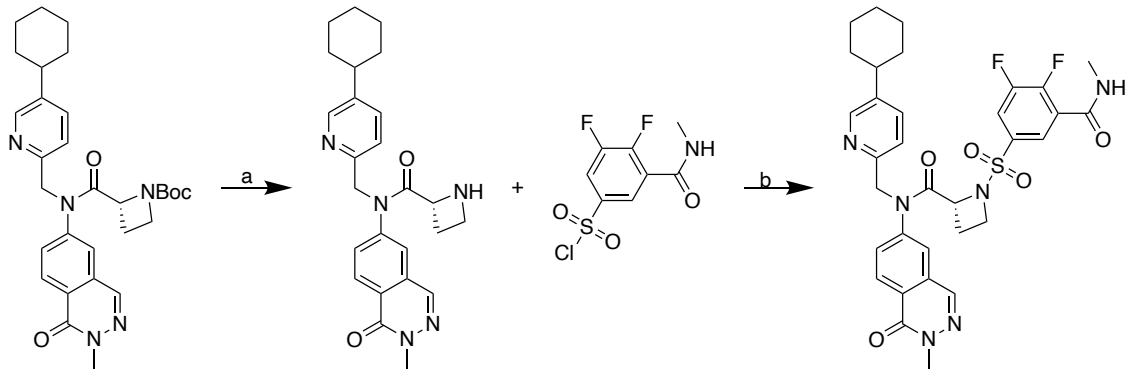
Scheme 18. Synthesis of **S3I-H299**[a]

[a]Reagents and Conditions: a) **39** (2.0 eq), Cs_2CO_3 (4.5 eq), NaI (0.2 eq), MeCN, 65 °C, 16 h, 47%; b) hydrolysis in work up c) MeMgBr (1.8 eq), **48** (2.0 eq), THF, 0 to 25 °C, 1 h, 45%; c) TFA (37.0 eq), DCM, rt, 1h; d) DIPEA (6.0 eq), **31** (1.3 eq), DCM, 0 to 25 °C, 2.5 h, 48% over two steps.

The synthesis of **S3I-H299** began in a unique fashion (**Scheme 18**). The S_N2 reaction between **45** and **39** led to deprotected aniline **47** instead of the anticipated product, **46**. This reaction gave the product, **47**, in 47% yield over two steps. This is similar to yields observed when the reactions were performed separately. It is unclear if **46** was deprotected during the course of the reaction or during the workup. It is possible not all of the cesium carbonate was neutralized in the work up, and under these basic aqueous conditions the trifluoroacetamide underwent rapid hydrolysis. The identity of **47** was confirmed by ^1H NMR, ^{19}F NMR and mass

spectrometry. Conditions that provide the deprotected aniline **47** directly would be useful. Aniline **47** was deprotonated with 1.8 equivalents of MeMgBr and then exposed to acid chloride, **48**. This gave **49** in 45% yield, a significant increase in yield over previous examples. The anilide **49** deprotected with excess TFA in DCM. The reaction mixture was then concentrated and used directly. To crude **50** was added excess Hünig's base followed by **31**. **S3I-H299** was obtained in 48% yield over two steps.

2.20 Synthesis of S3I-H308



Scheme 19. Synthesis of **S3I-H308**[a]

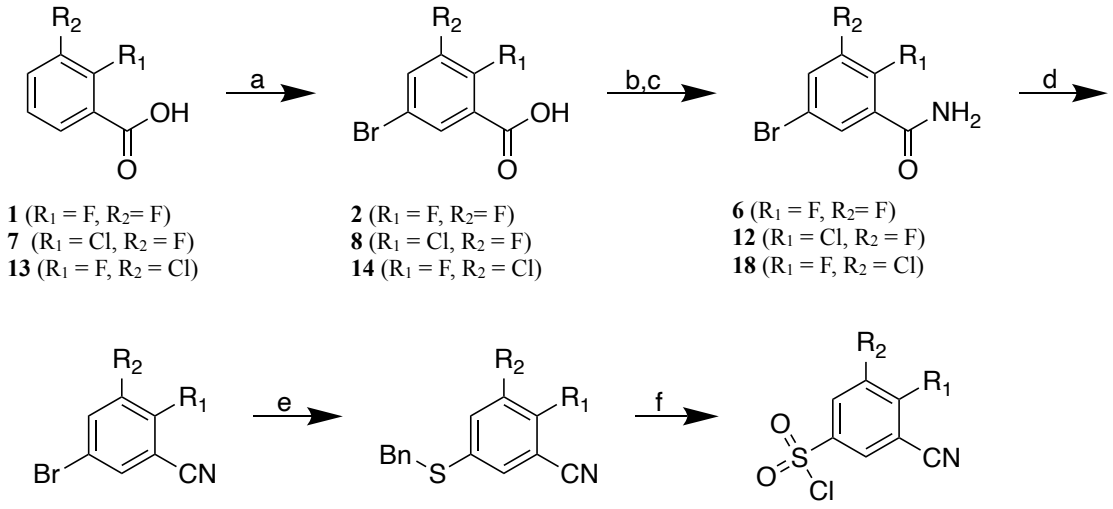
[a]Reagents and Conditions: a) TFA (37.0 eq), **34** (1.3 eq), DCM, rt, 1h; b) DIPEA (6.0 eq), DCM, **34** (1.3 eq), 0 to 25 °C, 2.5 h, 25% over two steps.

The synthesis of **S3I-H308** began with the deprotection of **71** (**Scheme 19**). The Boc group in **71** was deprotected with excess TFA in DCM to give **72**. Exposure of product **72** to Hünig's base followed by addition of **34** in a solution of DCM provided **S3I-H308** in 25% yield over two steps.

2.21 Summary of Synthetic Routes to Aryl Sulfonyl Chlorides

To start the screening of targets a stockpile of over eight grams of **6** was needed to supply the group and the effort described in this thesis. When the Tius Group received plasma stability data that suggested modification to the aryl sulfonamide was necessary it was envisioned that the sulfonyl chloride building blocks could all be made with small modifications of the common synthetic route developed for the difluorocyano sulfonyl chloride (**Scheme 20**). These modifications took the form of three routes. For compounds not containing a bromine an initial bromination step was necessary using NBS and sulfuric acid. The carboxylic acids were converted to the acid chloride, and then reacted with the appropriate amine to produce an amide. When compounds incorporated a nitrile, the amide was then dehydrated. The compounds were then coupled with benzyl mercaptan using the Itoh reaction to provide the thioether.[32] Finally, an oxidation was performed that led to the sulfonyl chloride.

Route A:



1 (R_1 = F, R_2= F)
7 (R_1 = Cl, R_2 = F)
13 (R_1 = F, R_2 = Cl)

2 (R_1 = F, R_2= F)
8 (R_1 = Cl, R_2 = F)
14 (R_1 = F, R_2 = Cl)

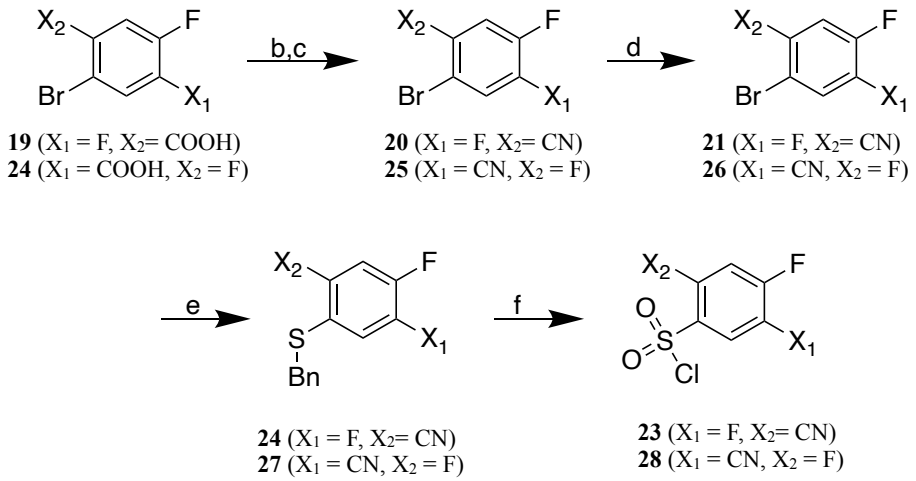
6 (R_1 = F, R_2= F)
12 (R_1 = Cl, R_2 = F)
18 (R_1 = F, R_2 = Cl)

4 (R_1 = F, R_2= F)
10 (R_1 = Cl, R_2 = F)
16 (R_1 = F, R_2 = Cl)

5 (R_1 = F, R_2= F)
11 (R_1 = Cl, R_2 = F)
17 (R_1 = F, R_2 = Cl)

3 (R_1 = F, R_2= F)
9 (R_1 = Cl, R_2 = F)
15 (R_1 = F, R_2 = Cl)

Route B:



19 (X_1 = F, X_2= COOH)
24 (X_1 = COOH, X_2 = F)

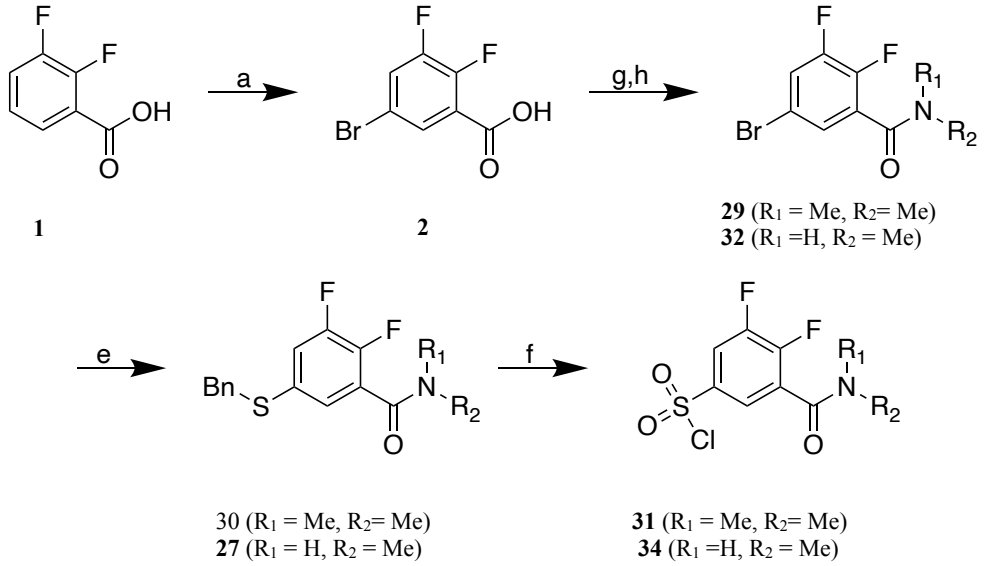
20 (X_1 = F, X_2= CN)
25 (X_1 = CN, X_2 = F)

21 (X_1 = F, X_2= CN)
26 (X_1 = CN, X_2 = F)

24 (X_1 = F, X_2= CN)
27 (X_1 = CN, X_2 = F)

23 (X_1 = F, X_2= CN)
28 (X_1 = CN, X_2 = F)

Route C:



1

2

29 (R_1 = Me, R_2= Me)
32 (R_1 =H, R_2 = Me)

30 (R_1 = Me, R_2= Me)
27 (R_1 = H, R_2 = Me)

31 (R_1 = Me, R_2= Me)
34 (R_1 =H, R_2 = Me)

Scheme 20. Proposed synthetic routes for the desired sulfonyl chlorides[a]

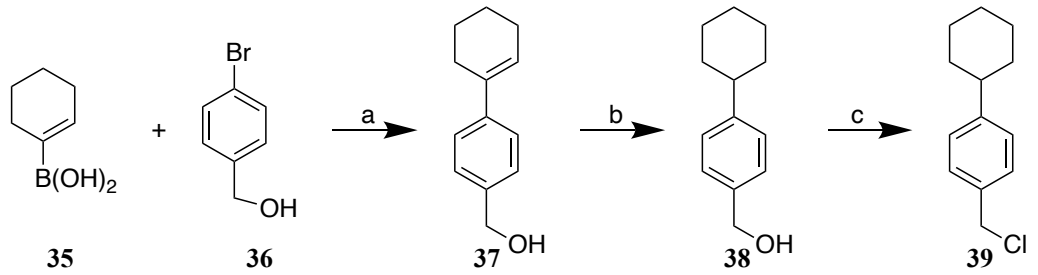
[a]Reagents and Conditions: a) H_2SO_4 (15.0 eq), NBS (1.5 eq), 60 °C, 3 h; b) oxalyl chloride (1.1 eq), DMF (cat.), DCM, 25 °C, 3 h; c) NH_4OH (excess), H_2O, 0 °C, 15 min; d) TFAA (1.1 eq), pyridine (2.2 eq), 1,4-dioxane, 0 to 25 °C, 4.5 h; e) $Pd_2(dba)_3$ (0.05 eq), Xantphos (0.1 eq), DIPEA (2.0 eq), BnSH (1.05 eq), 1,4-dioxane, reflux, 19 h; f) glacial acetic acid (4.5 eq), HPLC H_2O (7.0 eq), 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione (2.0 eq), MeCN, 0 to 25 °C, 1 h; g) oxalyl chloride (1.1 eq), DMF (cat.), DCM, 25 °C, 3 h; h) NR_2 (excess), H_2O, 0 °C, 15 min.

2.22 Synthetic Strategies for Production of STAT3 Inhibitors

Several convergent synthetic routes were developed by the group in order to readily introduce slight alterations in the structure in the search for optimum pharmacokinetics. Due to its continuous use, large amounts of **39** were prepared for the synthesis of inhibitors (**Scheme 21**). The cross coupling of **35** with **36** proceeded in excellent yield, 95%. The reduction **37** with Adams catalyst under an atmosphere of hydrogen gave **38** in 97%. Conversion of **38** to **39** was accomplished using thionyl chloride to give **39** in quantitative yield. The product could be stored as a solution of the free base in toluene or as the hydrochloride salt.

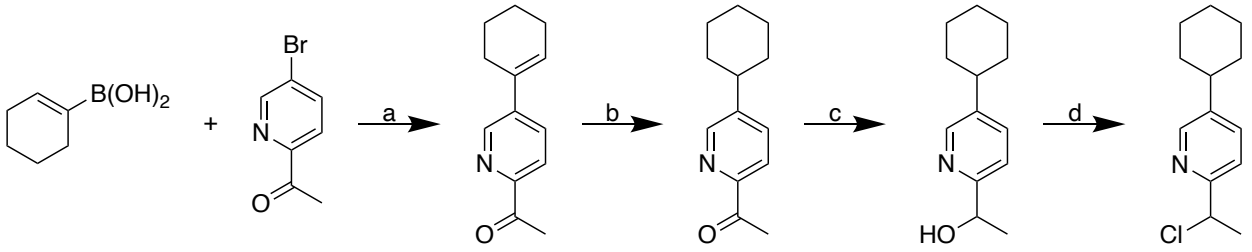
When the target included alkyl substitution at the benzylic position it was necessary to synthesize the appropriate alkyl chloride via a route similar to the one used to prepare **39** (**Scheme 21**). Coupling of **41** with cyclohex-1-en-1-ylboronic acid followed by reduction with Adams catalyst under an atmosphere of hydrogen afforded **42**. Reduction of **42** with sodium borohydride in methanol at 0 ˚C gave **43**, and the subsequent reaction of **43** with thionyl chloride gave **44**.

RouteA:



35 **36** **37** **38** **39**

Route B:



35 **40** **41** **42** **43** **44**

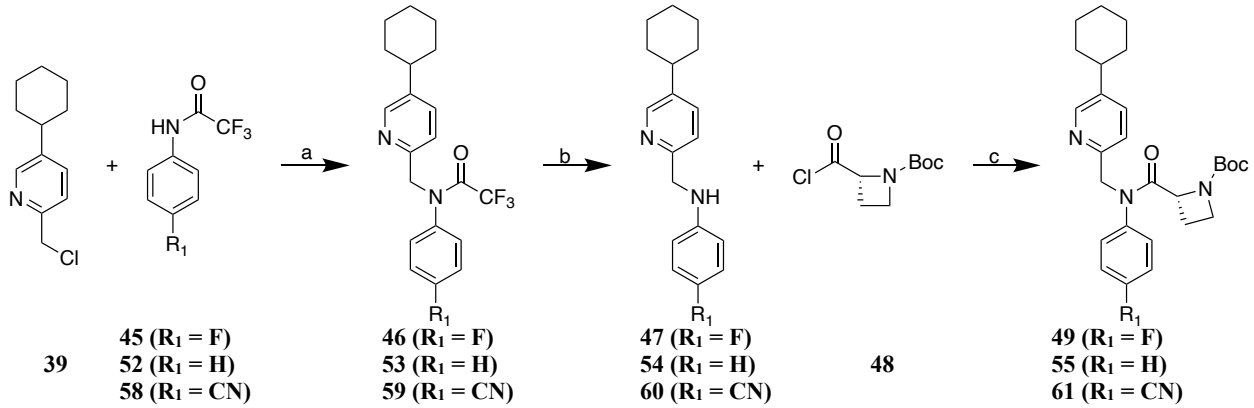
Scheme 21. Synthetic route to alkyl chlorides[a]

[a]Reagents and Conditions: Route A - a) SPhos (0.1 eq), $Pd(OAc)_2$ (0.05 eq), K_3PO_4 (2 eq), H_2O (2.0 eq), THF, 40 ˚C, 24 h; b) H_2, PtO_2 (10% by weight), 1:1 EtOAc:MeOH, rt, 24 h; c) d) $SOCl_2$ (1.2 eq), DCM, rt, 3 h. Route B - a) SPhos (0.1 eq), $Pd(OAc)_2$ (0.05 eq), K_3PO_4 (2.0 eq), H_2O (2.0 eq), THF, 40 ˚C, 24 h; b) H_2, PtO_2 (10% by weight), 1:1 EtOAc:MeOH, rt, 24 h; c) $NaBH_4$ (5.0 eq), MeOH, 0 ˚C, 3 h; d) $SOCl_2$ (1.2 eq), DCM, rt, 3 h.

It was envisioned that anilines (**45**, **52**, **58**) would be used as starting materials for the synthesis of the inhibitors described in this thesis (**Scheme 22**). Anilines were protected using TFAA and pyridine to give the trifluoroacetamide. The trifluoroacetamides would then be used in an S_N2 reaction with **39**. In **Route A** the trifluoroacetamide would then be deprotected to the aniline using K_2CO_3 in MeOH/THF(**Scheme 22**). The aniline would then be converted to the anilide using MeMgBr and (*R*)-tert-butyl-2-(chlorocarbonyl)azetidine-1-carboxylate. The anilide would be deprotected with TFA to provide the amine. Addition of DIPEA and the appropriate sulfonyl chloride would give the target.

For compounds **S3I-H247** and **S3I-H287** containing the pentafluorobenzenesulfonamide moiety, the synthesis would start with an S_N2 reaction of **45** with **39** or **44** (**Scheme 22**; **Route B**). The resulting trifluoroacetamide would be deprotected using the same conditions in **Route A**. The reaction of the aniline with MeMgBr and **66** would afford the target.

Route A:



39

45 (R_1 = F)
52 (R_1 = H)
58 (R_1 = CN)

46 (R_1 = F)
53 (R_1 = H)
59 (R_1 = CN)

47 (R_1 = F)
54 (R_1 = H)
60 (R_1 = CN)

48

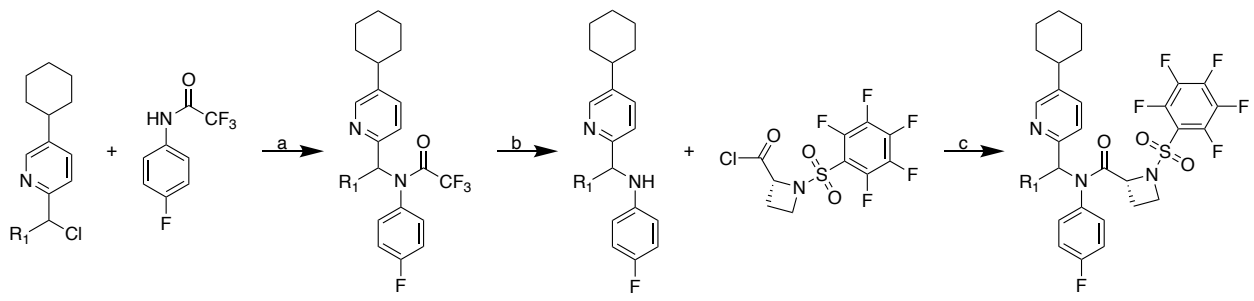
49 (R_1 = F)
55 (R_1 = H)
61 (R_1 = CN)

50 (R_1 = F)
56 (R_1 = H)
62 (R_1 = CN)

51 (R_1 = F, R_2)
57 (R_1 = H, R_2)
63 (R_1 = CN, R_2)

R_2 = various groups

Route B:



44 (R_1 = Me)
39 (R_1 = H)

45

64 (R_1 = Me)
46 (R_1 = H)

65 (R_1 = Me)
47 (R_1 = H)

66

S3I-H287 (R_1 = Me)
S3I-H147 (R_1 = H)

Scheme 22. Illustrates the two synthetic routes for the preparation of target compounds[a]

[a]Reagent and Conditions: Route A- a) **39** (2.0 eq), Cs_2CO_3 (4.5 eq), NaI (0.2 eq), MeCN, 60 ˚C, 16 h; b) K_2CO_3 (2.0 eq), 1:1 THF:MeOH, 3 h; c) MeMgBr (2.5 eq), **48** (2.0 eq), THF, 0 to 25 ˚C, 1 h; d) TFA (37.0 eq), DCM, rt, 1h; e) DIPEA (6.0 eq), DCM, 0 to 25 ˚C, 2.5 h. Route B - a) NaI (0.2 eq), K_2CO_3 (2.0 eq), MeCN, 60 ˚C, 16 h; b) K_2CO_3 (2.0 eq), 1:1 THF:MeOH, rt, 24 h; c) MeMgBr (2.5 eq), **66** (2.0 eq), THF, 0 to 25 ˚C, 1 h.

The thirteen inhibitors **S3I-H240, S3I-H241, S3I-H247, S3I-H254, S3I-H261, S3I-H265, S3I-H270, S3I-H275, S3I-H287, S3I-H289, S3I-H296, S3I-H299,** and **S3I-H308** were made using the Tius Group's convergent synthetic methods (Chapter 1; **Figure 11**). The inhibitors were characterized by ^{1}H NMR, ^{19}F NMR, and by HRMS/LCMS to determine that the purity of all materials submitted for biological evaluation was at least 95%. After characterization of the inhibitors they were sent to the Turkson Group for biological testing for STAT3 inhibitory activity via EMSA. Several of the compounds were also tested in MDA-231 to determine their potency in cancer cells. The results of the biological studies will be discussed in Chapter 3.

One of the most important features discussed in Chapter 2 is the modular synthesis of the STAT3 inhibitors and the sulfonyl chlorides (**Scheme 20**; **Scheme 22**). The approach of combining the various fragments of the inhibitors in different ways gives the group the ability to rapidly develop new analogues. While the Tius Group has already established synthetic routes, it was hypothesized that small modifications could be made in order to improve syntheses of targets.

In order to bring an inhibitor to clinical trials relatively large amounts of any lead compound would be required. In order to limit obstacles in any scale up, silica gel chromatography was avoided when possible. Purification steps limit scalability, and could be an added obstacle if large quantities of an inhibitor were required. The synthetic methods discussed in Chapter 2 removed two purifications by silica gel chromatography that were described in reports by previous group members. This cuts the number of purifications in production of most inhibitors from five to three. Each chromatographic separation diminishes yield because of mechanical losses, costs time, and money.

Additionally, the reaction to make the anilide in many of the syntheses was a troublesome step. The identification of contaminants in reaction solvent, followed by optimization of the equivalents of Grignard reagent solved the problem, and led to greatly increased yields. This represented the only reaction that was optimized significantly. Most of the other reactions were high yielding with yields of 65% or higher and were capable of producing material rapidly and on scale. Reactions that were needed only once in order to prepare one specific inhibitor were not optimized even if they were low yielding.

Chapter 3

Results and Conclusions

In Chapter 1 of this thesis, the important role that STAT3 plays in the initiation and progression of various carcinomas was discussed to demonstrate the need for the targeted inhibition of aberrantly activated STAT3. Biologists demonstrated the utility of previously developed STAT3 inhibitors by showing they reduced the tumor volume of mouse xenografts. The small molecule STAT3 inhibitors (**SH5-07**, **SH4-54**) produced by the Tius Group are orally bioavailable and inhibited tumor growth while displaying no noticeable signs of toxicity to the host. The mice maintained their weights, developed no overt changes to their liver or kidneys, and retained their activity levels.

In order for the compounds to advance to clinical studies in humans it was necessary to improve the current lead compounds' potency, stability, and selectivity. At the inception of this work it appeared that the difluorocyanobenzene motif would provide the desired potency, while also having selectivity for cancerous cell lines. However, it became obvious that the increased potency came at the cost of a loss of discrimination between nucleophiles. For the potency of the compounds to be high, the compound must react with a cysteine residue in the DBD of STAT3. However, if the compound is so reactive that it is consumed by indiscriminate reactions with plasma proteins or with GSH, it won't be of any use as a cancer treatment. The plasma stability of compounds containing the difluorocyanobenze moiety is not high enough for them to be viable as a cancer treatment. This provided the impetus to explore new motifs in order to better understand the SAR of region R_4 of the STAT3 inhibitors.

The groups that were used consistently through production of the thirteen inhibitors were: the pyridine unit in R_2, the cyclohexyl motif in R_3, and the (*R*)-azetidine linker (**Figure 11**). This combination of groups was the result of extensive SAR done by the Tius group, and has been the most used combination in novel inhibitors (**Table 1**, **Table 2**, **Table 3**). A majority of the compounds contained simple aryl units in R_1, such as phenyl, 4-fluorophenyl, or 4-cyanophenyl in order to readily make comparisons with compounds screened previously containing these groups.

The new compounds focused on variations to region R_4, which required the synthesis of unique sulfonyl chlorides, in particular constitutional isomers of the difluorocyano compounds. In some compounds the nitrile of the difluorocyano was replaced by an amide, and in others the

fluorine atoms at C25 and C26 were sequentially replaced with a chlorine atom (**Scheme 9**). It was hoped that a compound would be developed with desirable potency, selectivity, and stability by modifying the R_4 of potent compounds that lacked metabolic stability. With that in mind the following STAT3 inhibitors were designed and subsequently synthesized **S3I-H240**, **S3I-H241**, **S3I-H247**, **S3I-H254**, **S3I-H261**, **S3I-H265**, **S3I-H270**, **S3I-H275**, **S3I-H287**, **S3I-H289**, **S3I-H296**, **S3I-H298**, **S3I-H308** (**Figure 12**).

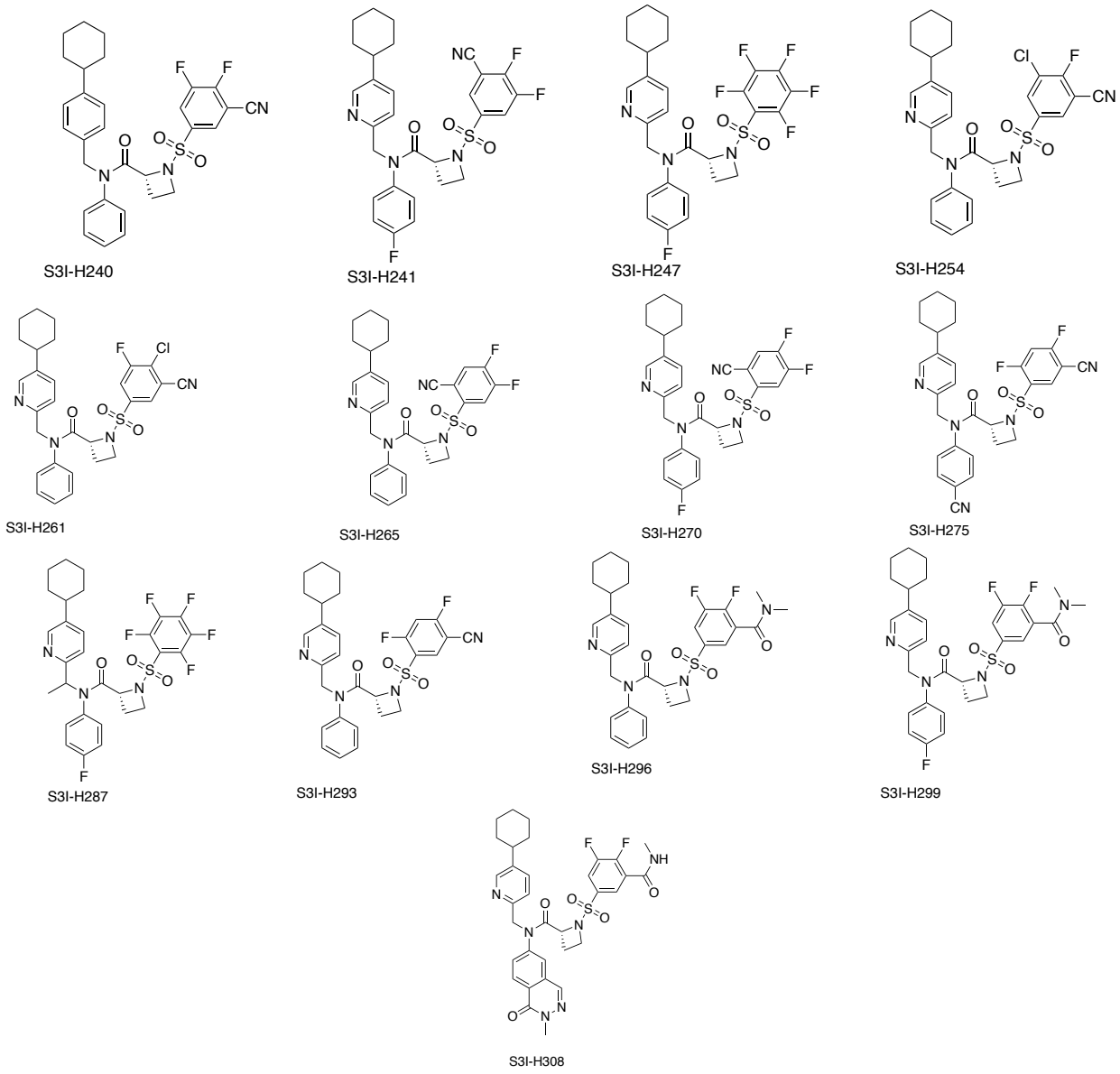


Figure 12. The STAT3 inhibitors prepared.

In Chapter 2 of this thesis the synthesis of each of the targeted small molecule inhibitors was discussed along with the synthesis of the seven unique sulfonyl chlorides (**Chapter 2**; **Scheme 1**; **Scheme 2**). The targets were prepared by convergent synthetic strategies that have broad functional group tolerance and substrate scope. These synthetic routes rely on a modular chemistry approach. The improvements to the syntheses were to avoid the purification of two intermediates by silica gel chromatography, as well as the optimization of the coupling of **39** with anilides such as **45**, **52**, and **58**. These improvements, along with the ability to produce large quantities of sulfonyl chlorides, enabled the production of inhibitors rapidly.

Most reactions discussed were high yielding. If reactions had low yields <65%, but were only performed once to produce an inhibitor they were generally not optimized. The main focus was on timely production of the inhibitors, not reaction optimization. Each of the targets was made, purified to >95% by LCMS, characterized spectroscopically and by HRMS before submission to the Turkson Group for testing to determine the STAT3 inhibitory activity via EMSA.

At the beginning of the work described in this thesis it appeared that the pentafluorobenzenesulfonamide was going to be abandoned and that the difluorocyanobenzene motif would provide the solution to selectivity, as well as potency problems. Because of the results of permeability studies discussed in Chapter 1 indicated that salicylic, benzoic, and hydroxamic acid groups severely limited cell permeability, simple aromatics were used in region R_1. Compound **S3I-H240** was compared to its analogue **S3I-H230** (EMSA IC_{50} 0.6 μM; MDA-231 IC_{50} 6.3 μM), which contains a pyridine in R_2, to determine what difference in potency and permeability a phenyl R_2 would make (**Figure 14**). **S3I-H240** (EMSA IC_{50} 3.3 μM; MDA-231 IC_{50} 7.0 μM) was less potent in both assays when compared to its parent. This result led to the abandonment of phenyl in favor of pyridyl for R_2.

The next compound synthesized, **S3I-H241**, was made with the hope of establishing cell permeability with simple motifs in region R_1 paired with the difluorocyano scaffold (**Figure 13**). Inhibitor **S3I-H241**'s (EMSA IC_{50} 2.5 μM; MDA-231 IC_{50} 2.6 μM) cellular assay indicates that the compound is cell permeable, and maintains its potency in cells. The metabolic stability data

suggested that the compound was not stable in plasma. Subsequently, the pentafluorobenzenesulfonamide was used in combination with 4-fluorophenyl in region R_1. This inhibitor, **S3I-H247** (IC_{50} >4 μM; MDA-231 IC_{50} 2.1 μM), was more stable in plasma, but was not very potent.

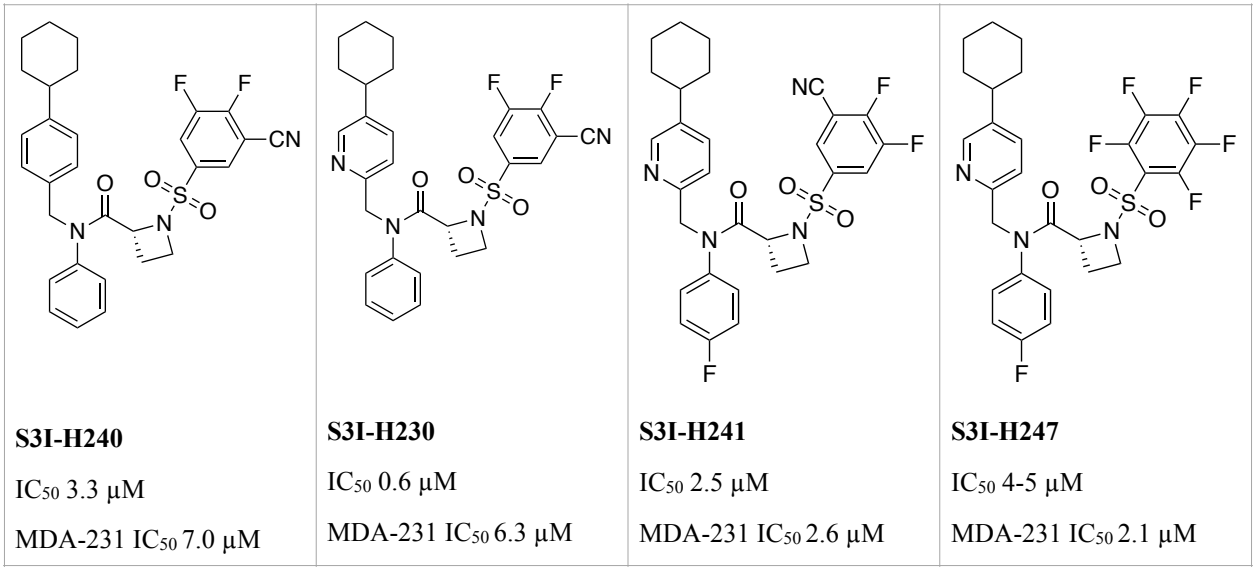
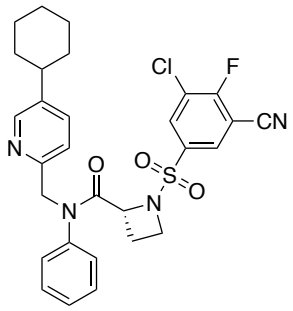


Figure 13. Structures of inhibitors and their EMSA and MDA-231 potency.

It was hypothesized that the fluorine atom para to the sulfonamide in the difluorobenzenesulfonamide containing inhibitors undergoes an S_NAr reaction with a cysteine residue in the STAT3 protein. In order to support this the fluorine atoms in **S3I-H230** (IC_{50} 0.6 μM; MDA-231 IC_{50} 6.3 μM) were sequentially replaced by a chlorine atom in new compounds (**Figure 14**). **S3I-H254** (IC_{50} 2.1 μM; MDA-231 IC_{50} 8.0 μM) is less potent in cells and in the EMSA relative to **S3I-H230**. This compound incorporated a novel group in R_4 and also provided evidence to support the mechanism of action of these STAT3 inhibitors. The low plasma stability of **S3I-H254** discouraged us from examining other inhibitors incorporating aryl groups in R_4 which contained a chlorine atom at C24 and a fluorine atom at C25 (**Figure 14**). Compound **S3I-H261** (IC_{50} >100 μM) also supports the proposed mechanism of action. The complete loss of potency after replacement of the para-fluorine atom with a chlorine atom demonstrates the

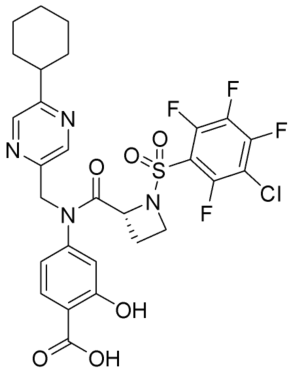
critical role of the fluorine atom at C25. The relatively small loss of potency when replacing the fluorine atom meta to the sulfonamide shows that this fluorine is not critical for potency. Replacing the fluorine atoms one at a time indicates that the fluorine para to the sulfonamide is the one reacting via an S_NAr reaction with a cysteine residue in STAT3.



S3I-H254

IC_{50} 2.1 μM

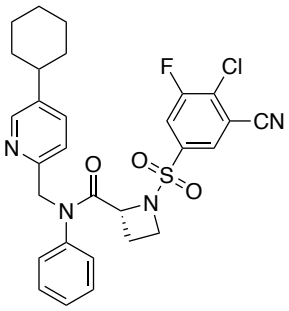
MDA-231 IC_{50} 8.0 μM



S3I-H194

IC_{50} 0.46 μM

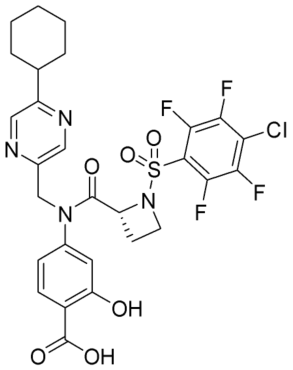
MDA-231 N/A



S3I-H261

IC_{50} >100 μM

MDA-231 N/A



S3I-H186

IC_{50} >100 μM

MDA-231 N/A

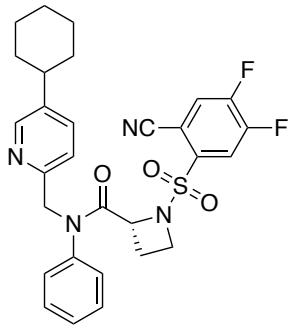
Figure 14. The structure of inhibitors introducing unique aryl groups in R_4, as well as their EMSA and MDA-231 potency.

The first constitutional isomer of **S3I-H230** (IC_{50} 0.6 μM) made was **S3I-H265** (IC_{50} >30 μM; **Figure 15**). This compound showed that placement of the nitrile group ortho to the sulfonamide results in poor potency. To confirm that this substitution pattern was ineffective the constitutional isomer of **S3I-H241** (IC_{50} 2.5 μM), **S3I-H270**, was made. Compound **S3I-H270 (**IC_{50} >20 μM) showed that further exploration of this substitution pattern was not going to be effective for the development drug-like compounds due to the relatively low potency of the compounds that contain it.

In the next compound of the series the fluorine atom was placed at C21 instead of C24. The target compound, **S3I-H275** (IC_{50} 1.49 μM; MDA-231 IC_{50} 8.86 μM), also contained a nitrile in region R_1 (**Figure 15**). When compared to the pentafluorobenzenesulfonamide **S3I-H266** (IC_{50} 10.1 μM; MDA-231 IC_{50} 3.2 μM) and the difluorocyanobenzesulfonamide **S3I-**

H260 (IC_{50} 2.4 μM; MDA-231 IC_{50} 2.24 μM) this compound has high EMSA potency, but lacks potency in MDA-231 cells. Subsequently, **S3I-H289** (IC_{50} 4-5 μM) was designed and prepared in order to provide evidence for viability of the new motif. The low potency provided little reason for further exploration.

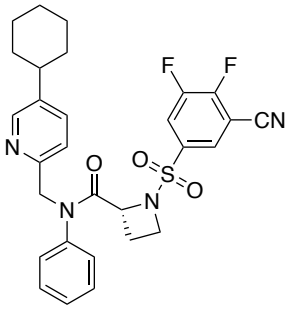
Hoping that alkyl substitution at C13 may increase potency in the compounds containing the pentafluorobenzenesulfonamide, **S3I-H287** (IC_{50} 2.8 μM; MDA-231 IC_{50} 2.28 μM) was designed and prepared. When compared to the non-alkylated analogue (**S3I-H247**) there was a small effect on potency (**Figure 15**). This compound was submitted for biological evaluation as a mixture of diastereomers.



S3I-H265

IC_{50} >30 μM

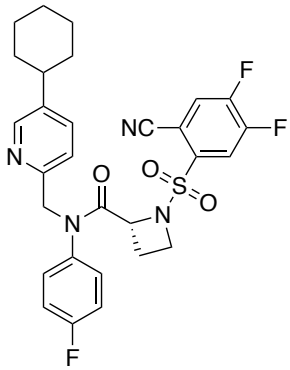
MDA-231 N/A



S3I-H230

IC_{50} 0.6 μM

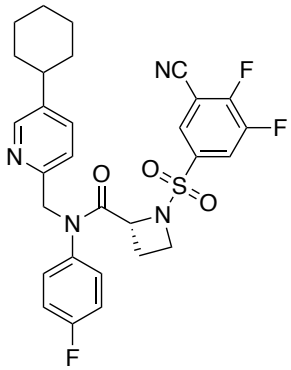
MDA-231 IC_{50} 6.3 μM



S3I-H270

IC_{50} >20 μM

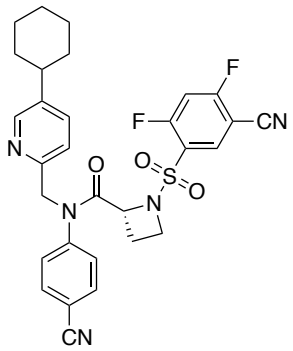
MDA-231 N/A



S3I-H241

IC_{50} 2.5 μM

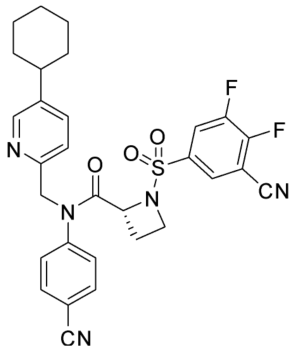
MDA-231 IC_{50} 2.6 μM



S3I-H275

IC_{50} 1.49 μM

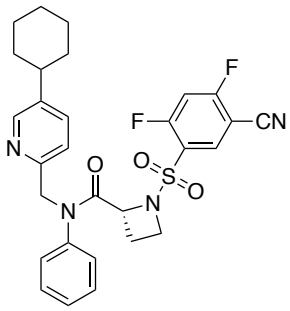
MDA-231 IC_{50} 8.8 μM



S3I-H260

IC_{50} 2.4 μM

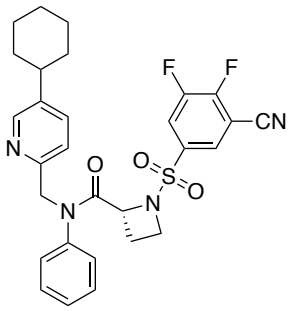
MDA-231 IC_{50} 2.2 μM



S3I-H289

IC_{50} 4-5 μM

MDA-231 N/A



S3I-H230

IC_{50} 0.6 μM

MDA-231 IC_{50} 6.3 μM

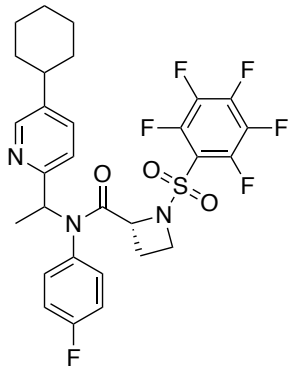
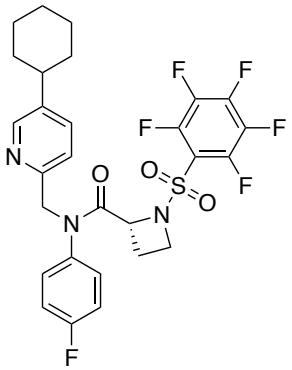
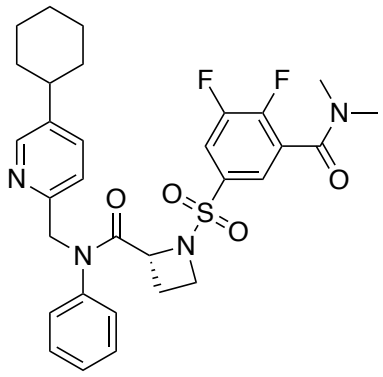
	
S3I-H287	**S3I-H247**
IC_{50} 2.8 μM	IC_{50} 4-5 μM
MDA-231 IC_{50} 2.3 μM	MDA-231 IC_{50} 2.1 μM

Figure 15. SAR of select inhibitors.

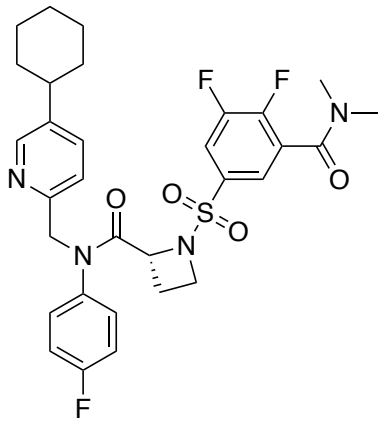
With the earlier results in hand, inhibitors **S3I-H296** and **S3I-H299** were both designed and prepared in order to determine the effect of a *N,N*-dimethylamide in R_4 (**Figure 16**). The compounds showed no significant activity. Each compound had an EMSA value greater than 20 μM. This was hypothesized to be due to the inability of the fluorine to effectively act as an electrophile within the binding pocket of the STAT3 protein due to steric crowding by the methyl groups on the amide. Subsequently mono-*N*-methyl amide **S3I-H308** was prepared, utilizing the phthalazinone scaffold in R_1 that had been determined to be promising. **S3I-H308** (IC_{50} 13.3 μM) inhibited STAT3 more effectively than **S3I-H296** and **S3I-H299**. Its effectiveness in MDA-231cells (8.3 μM) was slightly more encouraging, but is not potent enough to be viable as a drug.



S3I-H296

IC_{50} > 20 μM

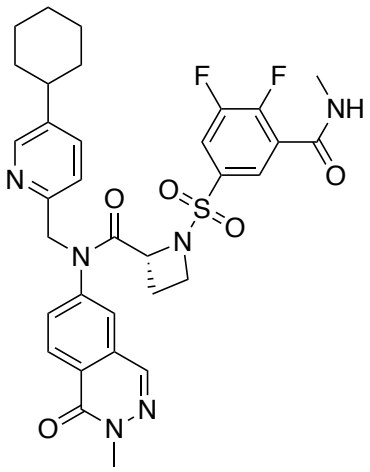
MDA-231 N/A



S3I-H299

IC_{50} >20 μM

MDA-231 N/A



S3I-H308

IC_{50} 13.3 μM

MDA-231 IC_{50} 8.3 μM

Figure 16. Targets with amides in R_4.

The contribution of the work herein can be summarized as follows: 1, production of thirteen small molecule STAT3 protein inhibitors for biological testing; 2, the optimization of standardized protocols for the production of novel or difficult to access sulfonyl chlorides; 3, removing several purification procedures in the synthesis of targets; 4, demonstrating the ability to use alkyl chloride **39** as the free base or as the hydrochloride salt. This work has enabled a reliable synthesis of STAT3 inhibitors with attenuated electrophilic reactivity. In addition, by eliminating unnecessary purification steps the cost in time and in money for the production of a target is decreased.

The most important conclusion drawn from this thesis relating to the SAR of the small molecule STAT3 protein inhibitors is: that in order to achieve the desired pharmacokinetics for a clinical candidate modifications will have to be made to the reactive site. Originally developed inhibitors **S3I-H240** and **S3I-H241** contain the difluorocyanobenzenesulfonamide. The difluorocyanobenzenesulfonamide scaffold is too reactive to be stable in plasma, while the pentafluorobenzenesulfonamide moiety largely lacks the desired potency and selectivity.

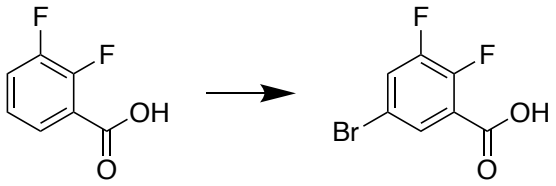
Attempts to optimize the difluorocyanobenzenesulfonamide by using constitutional isomers in inhibitors **S3I-H265**, **S3I-H270**, **S3I-H275**, and **S3I-H289** indicated through their potency and lack of plasma stability that they would be unsuitable for further study. Inhibitors **S3I-H254**, **S3I-H261** incorporated completely novel substitution patterns and a chlorine substituent into R_4. The activities of these inhibitors in EMSA supported the proposed mechanism of action. Inhibitors **S3I-H296**, **S3I-H299**, and **S3I-H308** were designed in order to determine whether the substitution of an amide for a nitrile group in R_4 would lead to potency and stability, however, the potency of these inhibitors was low. An attempt was made to improve the qualities of the pentafluorobenzenesulfonamide by synthesizing **S3I-H287**. The activity of this compound was only slightly higher than that of **S3I-H247** its parent.

The results of the SAR analysis on compounds in this thesis indicate that in order for a suitable drug-like candidate to be found, the electrophilic reactivity of the arylsulfonyl group in R_4 will require further adjustment. The current compounds appear to be unsuitable for consideration as clinical candidates, due to the trade off between potency and metabolic stability.

Experimental

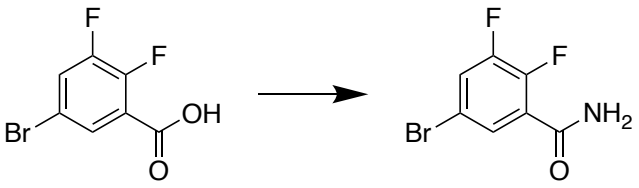
General

^{1}H NMR, and ^{19}F NMR were recorded on an Agilent DD2 spectrometer at 300 MHz (^{1}H), 75 MHz (^{13}C), 282 MHz (^{19}F) or on a Varian Unity Inova spectrometer at 500 MHz (^{1}H) and 487 MHz (^{19}F). Chemical shifts (δ) are reported in parts per million (ppm) and are referenced to the solvent, i.e. 7.26/77.2 for $CDCl_3$. Multiplicities are listed as: s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), t (triplet), q (quartet), quint (quintet), or any combination thereof, or m (multiplet). Coupling constants (*J*) are reported in Hertz (Hz). Thin-Layer Chromatography (TLC) was performed on Silicycle TLC glass plates, 250 μM, pore size 60 Å. Flash column chromatography was performed on Siliflash silica gel, 230-400 mesh. Preparative thin-layer chromatography was performed on Analtech uniplate prep TLC plates, 1000 μm. All moisture sensitive reactions were performed under a static atmosphere of argon in oven-dried or flame-dried glassware equipped with a stir bar with magnetic stirring. Purity and homogeneity of all materials was determined to be at least 95% from TLC and LCMS when applicable. Final compounds prepared for biological examination were purified to 95% and evaluated at 254 nm by C18 reverse phase HPLC using an Agilent 6410 triple quadrupole LCMS. High resolution mass spectrometric data were obtained on an Agilent LC-MS TOF with ESI ionization in positive mode. All compounds not fully characterized had either been prepared previously or were a component of a mixture that was purified at a later stage.



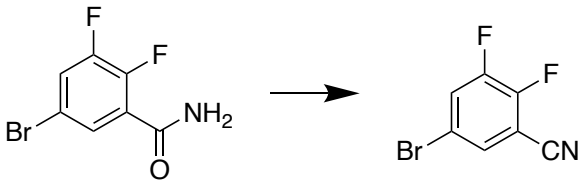
1 **2**

Synthesis of 2. To 2,3-difluorobenzoic acid (7.0 g, 44.3 mmol, 1.0 eq) was added concentrated H_2SO_4 (36 mL, 673.0 mmol, 15.0 eq) and *N*-bromosuccinimide (8.2 g, 46.5 mmol, 1.05 eq). The reaction mixture was heated at 60 ˚C for three hours. The reaction was allowed to cool to room temperature and poured onto ice. This mixture was allowed to stir at room temperature for five minutes, and filtered. The solid was washed with water. The solid was dissolved in ethyl acetate and the organic phase was extracted with 3M sodium hydroxide (x1). The aqueous phase was acidified to pH 2. The aqueous phase was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated to obtain 5-bromo-2,3-difluorobenzoic acid as a mixture due to a lack of regiospecificity in the bromination (8.60 g).



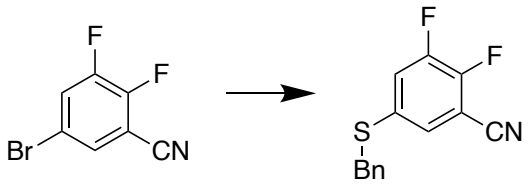
2 **3**

Synthesis of 3. To a solution of crude 5-bromo-2,3-difluorobenzoic acid (7.8 g, 32.9 mmol, 1.0 eq) in dichloromethane (60 mL) was added oxalyl chloride (4.2 mL, 50.0 mmol, 1.5 eq) followed by DMF (4-6 drops, cat.). The mixture was allowed to stir at room temperature for 2.5 hours and the solvent was removed *in vacuo*. The residue was dissolved in acetonitrile (30 mL), and the solution was poured onto ice cold concentrated ammonium hydroxide (318 mL). The mixture was allowed to warm to room temperature and stirred for 15 minutes. Water was added to the mixture, and it was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, and dried over sodium sulfate. After filtration, the solvent was removed *in vacuo* to afford crude 5-bromo-2,3-difluorobenzamide (7.50 g).



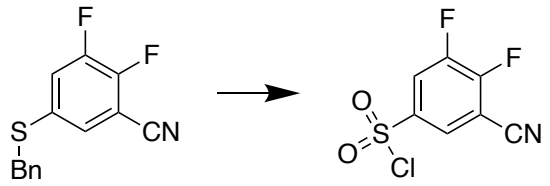
3 **4**

Synthesis of 4. To a solution of crude 5-bromo-2,3-difluorobenzamide (6.84 g, 29.0 mmol, 1.0 eq) in dioxane (45 mL) was added pyridine (4.7 mL, 58.4 mmol, 2.0 eq). The mixture was cooled in an ice-water bath, and trifluoroacetic anhydride (4.4 mL, 32.0 mmol, 1.1 eq) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for four and a half hours. The mixture was poured onto water, and extracted with ethyl acetate (x3). The combined organic extracts were washed with sodium bicarbonate, water, brine, and dried over sodium sulfate. After filtration, the solvent was removed *in vacuo* to afford crude 5-bromo-2,3-difluorobenzonitrile (5.70 g).



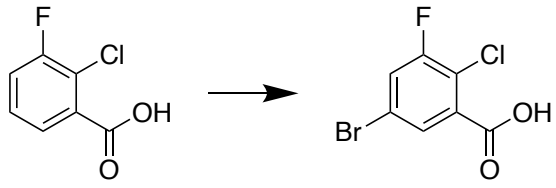
4 **5**

Synthesis of 5. To crude 5-bromo-2,3-difluorobenzonitrile (9.0 g, 41.2 mmol, 1.0 eq) was added $Pd_2(dba)_3$ (0.94 g, 1.0 mmol, 0.025 eq) and Xantphos (1.18 g, 2.0 mmol, 0.05 eq). The reaction vessel was evacuated and flushed with argon (x3). The solids were dissolved in dioxane (100 mL). Hünig's base (14.1 mL, 81.1 mmol, 2.0 eq) followed by benzyl mercaptan (5.0 mL, 42.6 mmol, 1.05 eq) were added. The reaction mixture was heated to reflux for 19 hours. After cooling to room temperature, water was added, and the mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting crude solid by silica gel chromatography (1:3 toluene/ hexanes) gave 2,3-difluoro-5-(phenylthio)benzonitrile as a white solid (7.00 g, 65% yield over five steps). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.3 — 7.2 (m, 7H), 4.13 (s, 2H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -132.8 (ddd, $J_{F\text{-}F}$ = 20.4 Hz, $J_{F\text{-}H}$ = 9.9, 1.4 Hz, 1F), -132.97 (ddd, $J_{F\text{-}F}$ = 20.4 Hz, $J_{F\text{-}H}$ = 7.4, 4.5 Hz, 1F)



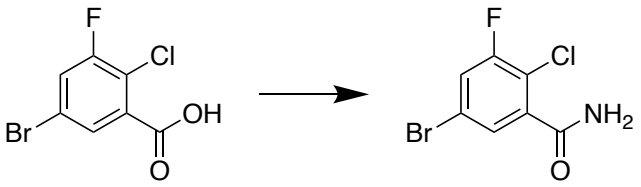
5 **6**

Synthesis of 6. To a solution of 2,3-difluoro-5-(phenylthio)benzonitrile (3.87 g, 14.8 mmol, 1.0 eq) in acetonitrile (80 mL) was added acetic acid (3.9 mL, 67.6 mmol, 4.5 eq) and HPLC-grade water (1.9 mL, 107.2 mmol, 7.0 eq). The mixture was cooled to 0 ˚C and 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione was added (6.88 g, 29.6 mmol, 2.0 eq). The ice bath was removed and the reaction mixture was stirred for one hour at room temperature. Water was added to the reaction, and the aqueous phase was extracted with ethyl acetate (x3). The combined organic extracts were washed with pH 7 buffer, water, brine, dried over sodium sulfate, and concentrated. The crude oil was filtered through a silica gel plug (96:4 hexanes/ethyl acetate) After concentration, the resulting oil was triturated with hexanes to afford compound **6** as a crystalline solid (2.50 g, 70% yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.2 — 8.13 (m, 2H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -116.8 (ddd, $J_{F\text{-}F}$ = 19.7 Hz, $J_{F\text{-}H}$ = 6.5, 4.7 Hz, 1F), -126.29 (ddd, $J_{F\text{-}F}$ = 19.7 Hz, $J_{F\text{-}H}$ = 8.9, 2.6 Hz, 1F)



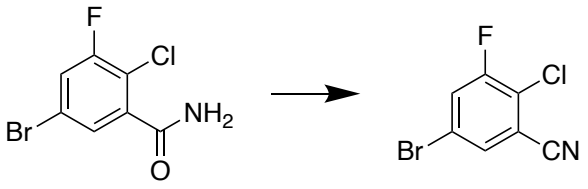
7 **8**

Synthesis of 8. To 2-chloro-3-fluorobenzoic acid (3.08 g, 17.6 mmol, 1.0 eq) was added concentrated H_2SO_4 (14.0 mL, 262.0 mmol, 15.0 eq) and *N*-bromosuccinimide (4.70 g, 26.4 mmol, 1.5 eq). The reaction mixture was heated at 60 ˚C for three hours. The reaction was allowed to cool to room temperature and poured onto ice. This mixture was allowed to stir at room temperature for five minutes, and filtered. The solid was washed with water. The solid was dissolved in ethyl acetate and the organic phase was washed with 3M sodium hydroxide (x1). The aqueous phase was acidified to pH 2. The aqueous phase was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. After concentration crude 5-bromo-2-chloro-3-fluorobenzoic acid was obtained as a white solid (3.20 g). This product was obtained as a mixture due to a lack of regioselectivity in the bromination.



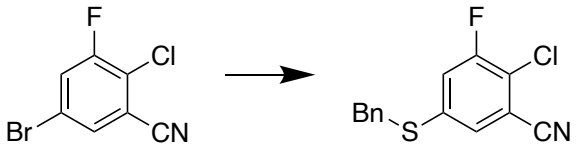
8 **9**

Synthesis of 9. To a solution of crude 5-bromo-2-chloro-3-fluorobenzoic acid (3.2 g, 12.6 mmol, 1.0 eq) in dichloromethane (40 mL) was added oxalyl chloride (1.6 mL, 18.75 mmol, 1.5 eq) followed by DMF (5 drops, cat). The reaction mixture was allowed to stir at room temperature for 2.5 hours and the reaction solvent was removed *in vacuo*. The residue was dissolved in acetonitrile (20 mL), and the solution was poured onto ice cold concentrated ammonium hydroxide (150 mL). The mixture was allowed to warm to room temperature and stirred for 15 minutes. Water was added to the mixture, and it was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated to obtain crude 5-bromo-2-chloro-3-fluorobenzamide as a white solid (2.91 g).



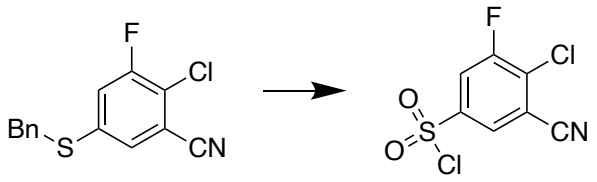
9 **10**

Synthesis of 10. To a solution of crude 5-bromo-2-chloro-3-fluorobenzamide (2.912 g, 11.55 mmol, 1.0 eq) in dioxane (50 mL) was added pyridine (1.9 mL, 23.5 mmol, 2.0 eq). The solution was cooled in an ice-water bath, and trifluoroacetic anhydride (1.8 mL, 12.9 mmol, 1.1 eq) was added dropwise. The reaction was allowed to warm to room temperature and stirred for 16 hours. The mixture was poured onto water, and extracted with ethyl acetate (x3). The organic extracts were washed with sodium bicarbonate. The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated to obtain crude 5-bromo-2-chloro-3-fluorobenzonitrile as a white solid (2.59 g).



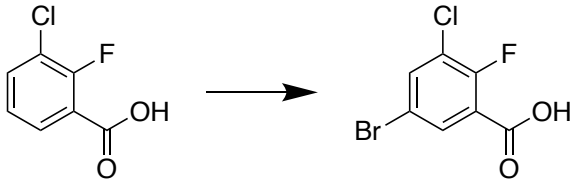
10 **11**

Synthesis of 11. To crude 5-bromo-2-chloro-3-fluorobenzonitrile (798 mg, 3.4 mmol, 1.0 eq) was added $Pd_2(dba)_3$ (77 mg, 0.08 mmol, 0.05 eq) and Xantphos (98 mg, 0.16 mmol, 0.1 eq). The reaction vessel was evacuated and flushed with argon (x3). The solids were dissolved in dioxane (10 mL). Subsequently Hünig's base (1.1 mL, 6.3 mmol, 1.9 eq), and benzyl mercaptan (0.4 mL, 3.5 mmol, 1.05 eq) were added. The reaction mixture was heated to reflux for 19 hours. After cooling to room temperature, water was added, and the mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over sodium sulfate, and concentrated. Purification of the crude solid by silica gel chromatography (1:3 toluene/ hexanes) afforded 2-chloro-3-fluoro-5-(phenylthio)benzonitrile as a white solid (155 mg, 5% yield over five steps). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 - 7.30 (m, 6H), 7.26 (dd, J_{F-H} = 8.9 Hz, J_{H-H} = 2.1 Hz, 1H), 4.17 (s, 2H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -110.25 (dd, J_{F-H} = 8.9, 1.1 Hz, 1F).



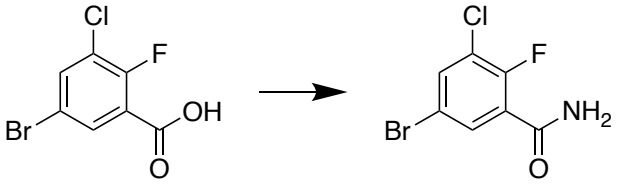
11 **12**

Synthesis of 12. To a solution of 2-chloro-3-fluoro-5-(phenylthio)benzonitrile (160 mg, 0.5 mmol, 1.0 eq) in acetonitrile (6 mL) was added acetic acid (0.15 mL, 2.6 mmol, 5.0 eq) and water (0.7 mL, 40 mmol, 8.0 eq). The mixture was cooled to 0 ˚C and 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione (260 mg, 1.1 mmol, 2.2 eq) was added. The ice bath was removed and the reaction mixture was stirred at room temperature for one hour. Water was added to the reaction and extracted with ethyl acetate (x3). The organic extracts were washed with pH 7 buffer, water, brine, dried over sodium sulfate, and concentrated. The crude oil was filtered through a silica gel plug (96:4 hexanes/ethyl acetate). After concentration, the resulting oil was triturated with hexanes to afford 3-cyano-4-chloro-5-fluorobenzenesulfonyl chloride as a white solid (60 mg, 40% yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (dd, J_{F-H} = 1.5 Hz, J_{H-H} = 2.2 Hz, 1H), 7.74 (dd, J_{F-H} = 7.1 Hz, J_{H-H} = 2.2 Hz, 1H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -108.38 (dd, J_{F-H} = 7.1, 1.5 Hz, 1F).



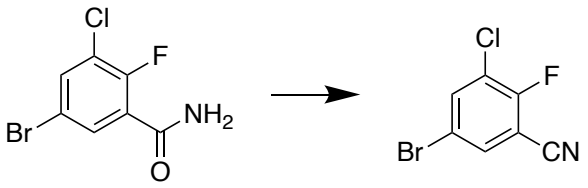
13 **14**

Synthesis of 14. To 2-fluoro-3-chlorobenzoic acid (1.5 g, 8.6 mmol, 1.0 eq) was added concentrated H_2SO_4 (7.0 mL, 131.3 mmol, 15.2 eq) and *N*-bromosuccinimide (1.6 g, 9.0 mmol, 1.05 eq). The reaction mixture was heated at 60 °C for three hours. The reaction mixture was allowed to cool to room temperature and poured onto ice water. The mixture was allowed to stir at room temperature for five minutes, and filtered. The solid was washed with water. The solid was dissolved in ethyl acetate and the organic phase was extracted with 3M sodium hydroxide (x1). The aqueous phase was acidified with acidified to pH 2. The aqueous phase was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated to obtain crude 5-bromo-2-fluoro-3-chlorobenzoic acid as a white solid (1.71 g). This product was obtained as a mixture as a result of the limited regioselectivity of the bromination. The impurities were removed at a later stage.



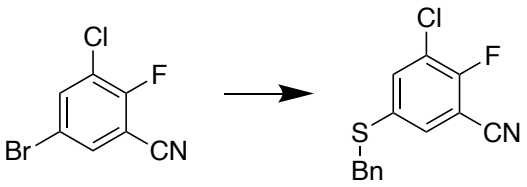
14 **15**

Synthesis of 15. To a solution of crude 5-bromo-2-fluoro-3-chlorobenzoic acid (3.165 g, 12.5 mmol, 1.0 eq) in dichloromethane (40 mL) was added oxalyl chloride (1.6 mL, 18.8 mmol, 1.5 eq) followed by DMF (5 drops, cat). The mixture was allowed to stir at room temperature for 2.5 hours and the solvent was removed *in vacuo*. The resulting residue was dissolved in acetonitrile (30 mL), and the solution was poured onto ice cold concentrated ammonium hydroxide (150 mL). The mixture was allowed to reach room temperature and stirred for 15 minutes. Water was added to the mixture, and it was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated to obtain crude 5-bromo-2-fluoro-3-chlorobenzamide (2.89 g).



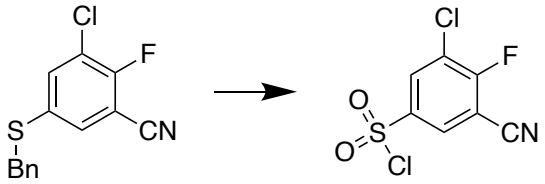
15 **16**

Synthesis of 16. To a solution of crude 5-bromo-2-fluoro-3-chlorobenzamide (3.05 g, 12.1 mmol, 1.0 eq) in dioxane (40 mL) was added pyridine (1.9 mL, 24.3 mmol, 2.0 eq). The solution was cooled using an ice-water bath, and trifluoroacetic anhydride (1.9 mL, 13.3 mmol, 1.1 eq) was added dropwise. The reaction was allowed to warm to room temperature and stirred for four and a half hours. The mixture was poured onto water, and extracted with ethyl acetate (x3). The organic combined extracts were washed with sodium bicarbonate, water, brine, dried over Na_2SO_4, and concentrated to obtain crude 5-bromo-2-fluoro-3-chlorobenzonitrile (2.26 g).



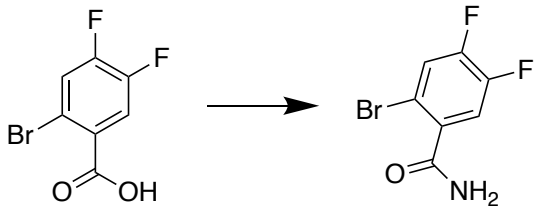
16 **17**

Synthesis of 17. To crude 5-bromo-2-fluoro-3-chlorobenzonitrile (640 mg, 2.7 mmol, 1.0 eq) was added $Pd_2(dba)_3$ (62 mg, 0.06 mmol, 0.05 eq) and Xantphos (78 mg, 0.12 mmol, 0.1 eq). The reaction vessel was evacuated and flushed with argon (x3). The solids were dissolved in dioxane (10 mL). Hünig's base (0.1 mL, 5.4 mmol, 2.0 eq) was added followed by benzyl mercaptan (0.3 mL, 2.8 mmol, 1.05 eq). The reaction mixture was heated to reflux for 19 hours. After cooling to room temperature, water was added, and the mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the crude solid by silica gel chromatography (1:3 toluene/hexanes) gave 2-fluoro-3-chloro-5-(phenylthio)benzonitrile as a white solid (340 mg, 24% yield over five steps). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (dd, J_{F-H} = 6.6 Hz, J_{H-H} = 2.3 Hz, 1H), 7.37 (dd, J_{F-H} = 5.2 Hz, J_{H-H} = 2.3 Hz, 1H), 7.35 - 7.24 (m, 5H), 4.12 (s, 2H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -110.86 (dd, J_{F-H} = 6.6, 5.2 Hz, 1F).



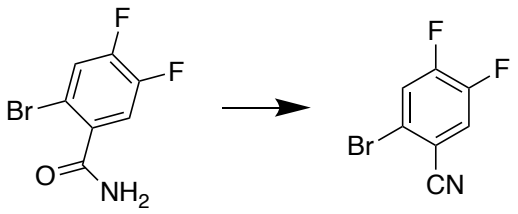
17 **18**

Synthesis of 18. To a solution of 2-fluoro-3-chloro-5-(phenylthio)benzonitrile (1.03 g, 3.7 mmol, 1.0 eq) in acetonitrile (30.0 mL) was added acetic acid (0.1 mL, 16.9 mmol, 4.5 eq) and water (0.5 mL, 26.8 mmol, 7.2 eq). The reaction mixture was cooled to 0 ˚C and 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione was added (1.726 g, 7.4 mmol, 2.0 eq). The ice bath was removed and the reaction mixture was stirred for one hour. Water was added to the reaction mixture, and the aqueous phase was extracted with ethyl acetate (x3). The combined organic extracts were washed with pH 7 buffer, water, brine, dried over Na_2SO_4, and concentrated. The crude oil was filtered through a silica gel plug (96:4 hexanes/ethyl acetate). After concentration, the resulting oil was triturated with hexanes to afford 3-chloro-5-cyano-4-fluorobenzenesulfonyl chloride as a crystalline white solid (342 mg, 42% yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (dd, $J_{F\text{-}H}$ = 6.1 Hz, $J_{H\text{-}H}$ = 2.3 Hz, 1H), 8.27 (dd, $J_{F\text{-}H}$ = 5.2 Hz, $J_{H\text{-}H}$ = 2.3 Hz, 1H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -95.35 (dd, $J_{F\text{-}H}$ = 6.1, 5.2 Hz, 1F).



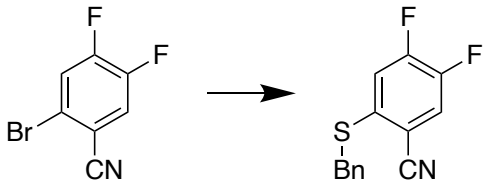
19 **20**

Synthesis of 20. To a solution of 2-bromo-4,5-difluorobenzoic acid (2.0 g, 8.43 mmol, 1.0 eq) in dichloromethane (30 mL) was added oxalyl chloride (1.0 mL, 11.6 mmol, 1.4 eq) followed by DMF (1 drop, cat). The mixture was allowed to stir at room temperature for 2.5 hours and then the solvent was removed *in vacuo*. The resulting residue was dissolved in acetonitrile (20 mL), and the solution was poured onto ice cold concentrated ammonium hydroxide (81 mL). The mixture was allowed to warm to room temperature and stirred for 15 minutes. Water was added to the mixture, and it was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated to obtain 2-bromo-4,5-difluorobenzamide (1.80 g, 91% yield). Spectroscopic Data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (ddd, J_{F-H} = 10.3, 8.3 Hz, J_{H-H} = 1.6 Hz, 1H), 7.48 (ddd, J_{F-H} = 9.3, 7.3 Hz, J_{H-H} = 1.6 Hz, 1H), 6.23 (s, 1H), 5.98 (s, 1H); ^{19}F NMR (471 MHz, $CDCl_3$) δ -130.73 (ddd, J_{F-F} = 21.2 Hz, J_{F-H} = 9.3, 8.3 Hz, 1F), -136.57 (ddd, J_{F-F} = 21.2 Hz, J_{F-H} = 10.3, 7.3 Hz, 1F).



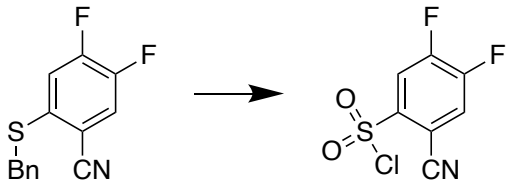
20 **21**

Synthesis of 21. To a solution of 2-bromo-4,5-difluorobenzamide (1.803 g, 7.63 mmol, 1.0 eq) in dioxane (15 mL) was added pyridine (1.2 mL, 14.9, 1.96 eq). The solution was cooled in an ice-water bath, and trifluoroacetic anhydride (1.4 mL, 9.9 mmol, 1.2 eq) was added dropwise. The reaction mixture was allowed to reach room temperature and stirred for four and a half hours. The mixture was poured onto water and was extracted with ethyl acetate (x3). The combined organic extracts were then washed with sodium bicarbonate (x1). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated to obtain 2-bromo-4,5-difluorobenzonitrile (1.45 g, 87% yield).



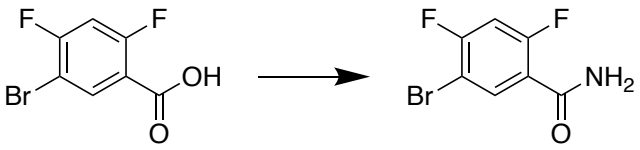
21 **22**

Synthesis of 22. To 2-bromo-4,5-difluorobenzonitrile (1.15 g, 5.3 mmol, 1.0 eq) was added $Pd_2(dba)_3$ (118 mg, 0.12 mmol, 0.05 eq) and Xantphos (152 mg, 0.24, 0.1 eq). The reaction vessel was evacuated and flushed with argon (x3). The solids were dissolved in dioxane (10 mL). Hünig's base (1.8 mL, 10.05 mmol, 1.9 eq) was added followed by benzyl mercaptan (0.6 mL, 5.5 mmol, 1.05 eq). The reaction mixture was heated to reflux for 19 hours. After cooling to room temperature, water was added, and the mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the crude solid by silica gel chromatography (1:3 toluene/hexanes) afforded 2-(benzylthio)-4,5-difluorobenzonitrile (737 mg, 55% yield). Spectroscopic Data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (ddd, J_{F-H} = 9.9, 7.4 Hz, J_{H-H} = 0.9 1H), 7.36 – 7.28 (m, 5H), 7.17 (ddd, J_{F-H} = 9.9, 6.6 Hz, J_{H-H} = 0.9 Hz, 1H), 4.20 (s, 2H); ^{19}F NMR (471 MHz, $CDCl_3$) δ -126.16 (ddd, J_{F-F} = 21.5 Hz, J_{F-H} = 9.9, 7.4 Hz, 1F), -136.23 (ddd, J_{F-F} = 21.5 Hz, J_{F-H} = 9.9, 6.6 Hz, 1F).



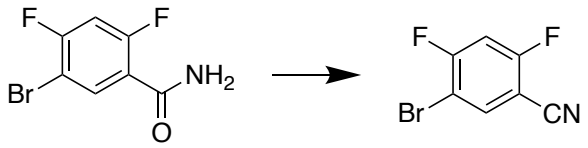
22 **23**

Synthesis of 23. To a solution of 2-(benzylthio)-4,5-difluorobenzonitrile (300 mg, 1.15 mmol, 1.0 eq) in acetonitrile (5 mL) was added acetic acid (0.3 mL, 5.25, 4.5 eq) and water (0.15 mL, 7.7 mmol, 6.7 eq). The mixture was cooled to 0 ˚C and 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione (520 mg, 2.24, 1.95 eq) was added. The ice-bath was removed and the reaction was stirred at room temperature for one hour. Water was added and the mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with pH 7 buffer, water, brine, dried over Na_2SO_4, and concentrated. The crude solid was filtered through a silica plug (96:4 hexanes/ ethyl acetate). After concentration, the resulting oil was triturated to afford 2-cyano-4,5-difluorobenzenesulfonyl chloride as a crystalline white solid (231 mg, 85% yield). Spectroscopic Data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (ddd, J_{F-H} = 9.5, 7.4 Hz, J_{H-H} = 2.2 Hz 1H), 7.86 (ddd, J_{F-H} = 9.1, 6.6 Hz, J_{H-H} = 2.1 Hz, 1H); ^{19}F NMR (471 MHz, $CDCl_3$) δ -121.12 (ddd, J_{F-F} = 20.7 Hz, J_{F-H} = 9.5, 6.6 Hz, 1F), -121.25 (ddd, J_{F-F} = 20.7 Hz, J_{F-H} = 9.1, 7.4 Hz, 1F).



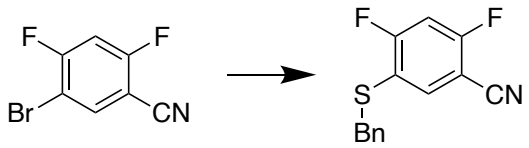
24 **25**

Synthesis of 25. To a solution of 5-bromo-2,4-difluorobenzoic acid (3.0 g, 12.6 mmol, 1.0 eq) in dichloromethane (50 mL) was added oxalyl chloride (1.5 mL, 17.8 mmol, 1.4 eq) followed by DMF (4 drops, cat). The mixture was allowed to stir at room temperature for 2.5 hours and the solvent was removed *in vacuo*. The resulting residue was dissolved in acetonitrile (20 mL), and the solution was poured onto ice-cold concentrated ammonium hydroxide (120 mL). The mixture was allowed to reach room temperature and stirred for 15 minutes. Water was added to the mixture, and it was extracted with ethyl acetate (x3). The combined organic extracts were then washed with water, brine, dried over Na_2SO_4, and concentrated to obtain 5-bromo-2,4-difluorobenzamide (2.78 g, 93%). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (dd, J_{F-H} = 7.5, 6.9 Hz 1H), 7.01 (dd, J_{F-H} = 9.3, 8.5 Hz, 1H), 6.60 (s, 1H), 6.03 (s, 1H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -92.45 (ddd, J_{F-F} = 11.6 Hz, J_{F-H} = 9.3, 6.9 Hz, 1F), -102.77 (ddd, J_{F-F} = 11.6 Hz, J_{F-H} = 7.5, 2.9 Hz, 1F).



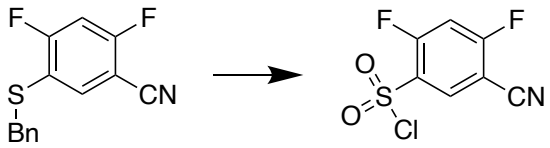
25 **26**

Synthesis of 26. To a solution of 5-bromo-2,4-difluorobenzamide (1.803 g, 7.6 mmol, 1.0 eq) in dioxane (15 mL) was added pyridine (1.2 mL, 14.9 mmol, 1.96 eq). The solution was cooled in an ice-water bath, and trifluoroacetic anhydride (1.4 mL, 9.99 mmol, 1.3 eq) was added drop-wise. The reaction mixture was allowed to warm to room temperature and stirred for four and a half hours. The mixture was poured onto water, and extracted with ethyl acetate (x3). The combined organic extracts were washed with sodium bicarbonate (x1). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated to obtain 5-bromo-2,4-difluorobenzonitrile (1.16 g, 97% yield).



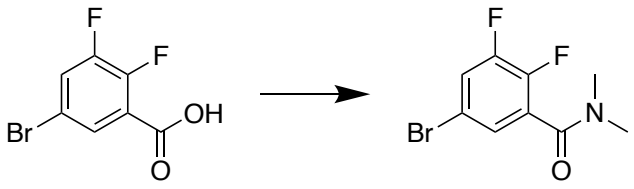
26 **27**

Synthesis of 27. To 2-bromo-4,5-difluorobenzonitrile (2.241 g, 10.27 mmol, 1 eq) was added $Pd_2(dba)_3$ (230 mg, 0.32 mmol, 0.05 eq) and Xantphos (296 mg, 0.64 mmol, 0.1 eq). The reaction vessel was evacuated and flushed with argon (x3). The solids were dissolved in dioxane (25 mL). Hünig's base (3.5 mL, 19.95 mmol, 1.95 eq) was added followed by benzyl mercaptan (1.3 mL, 10.77 mmol, 1.05 eq). The reaction mixture was heated to reflux for 19 hours. After cooling to room temperature, water was added, and the mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the crude solid by silica gel chromatography (1:3 hexane/toluene) gave 2-(benzylthio)-4,5-difluorobenzonitrile (1.96 g, 73% yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (dd, J_{F-H} = 7.8, 7.0 Hz, 1H), 7.35 – 7.28 (m, 3H), 7.26 – 7.20 (m, 2H), 7.04 – 6.95 (dd, J_{F-H} = 9.0, 8.5 Hz, 1H), 4.09 (s, 2H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -92.45 (ddd, J_{F-F} = 13.0 Hz, J_{F-H} = 9.0, 7.0 Hz, 1F), -102.77 (ddd, J_{F-F} = 13.0 Hz, J_{F-H} = 8.5, 7.8 Hz, 1F).



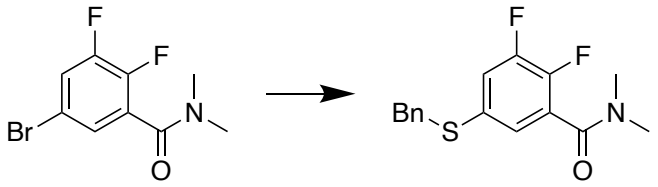
27 **28**

Synthesis of 28. To a solution of 2-(benzylthio)-4,5-difluorobenzonitrile (902 mg, 3.45 mmol, 1.0 eq) in acetonitrile (15 mL) was added acetic acid (0.90 mL, 15.78 mmol, 4.5 eq) and water (0.5 mL, 24.9 mmol, 7.2 eq). The mixture was cooled to 0 ˚C and 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione was added (1.6 g, 6.89 mmol, 2.0 eq). The ice-bath was removed and the reaction mixture was stirred for one hour. Water was added to the reaction mixture, and the aqueous phase was extracted with ethyl acetate (x3). The combined organic extracts were washed with pH 7 buffer, water, brine, dried over Na_2SO_4, and concentrated. The crude oil was filtered through a silica gel plug (96:4 hexanes-ethyl acetate). After concentration, the resulting oil was triturated with hexanes to afford 2-cyano-4,5-difluorobenzenesulfonyl chloride as a crystalline solid (505 mg, 62% yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (dd, J_{F-H} = 7.3, 6.6 Hz, 1H), 7.36 (dd, J_{F-H} = 8.9, 8.3 Hz, 1H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -87.90 (ddd, J_{F-F} = 19.6 Hz, J_{F-H} = 8.3, 6.6 Hz, 1F), -90.77 (ddd, J_{F-F} = 19.6 Hz, J_{F-H} = 8.9, 7.3 Hz, 1F).



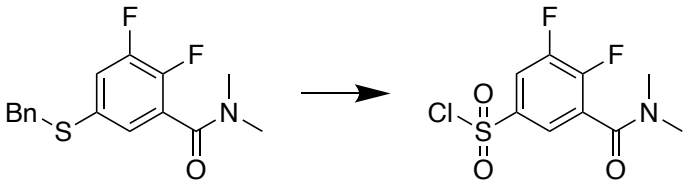
2 **29**

Synthesis of 29. To a solution of crude 5-bromo-2,3-difluorobenzoic acid (4.29 g, 18.1 mmol, 1.0 eq) in dichloromethane (30 mL) was added oxalyl chloride (2.2 mL, 25.25 mmol, 1.4 eq) followed by DMF (4-6 drops, cat). The mixture was allowed to stir at room temperature for 2.5 hours and then the reaction solvent was removed *in vacuo*. The resulting residue was dissolved in acetonitrile (20 mL), and the solution was poured onto dimethyl amine (excess) in acetonitrile (10 mL). The mixture was allowed to reach room temperature and stirred for 15 minutes. Water was added to the mixture, and the mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated to obtain crude 5-bromo-2,3-difluoro-*N*,*N*-dimethylbenzamide (3.52 g).



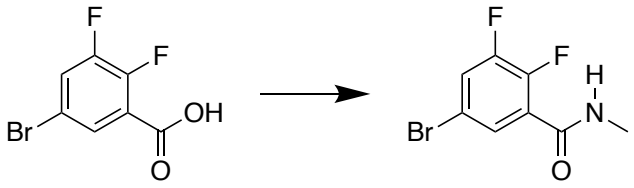
29 **30**

Synthesis of 30. To crude 5-bromo-2,3-difluoro-*N*,*N*-dimethylbenzamide (725 mg, 2.73 mmol, 1.0 eq) was added $Pd_2(dba)_3$ (128 mg, 0.13 mmol, 0.05 eq) and Xantphos (161 mg, 0.26 mmol, 0.1 eq). The reaction vessel was evacuated and flushed with argon (x3). The solids were dissolved in dioxane (10 mL), and *i*-Pr_2NEt (0.1 mL, 5.59 mmol, 2.0 eq) was added, followed by benzyl mercaptan (0.4 mL, 3.6 mmol, 1.2 eq). The reaction mixture was heated at reflux for 19 hours. After cooling to room temperature, water was added, and the mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the crude solid by silica gel chromatography (1:4 ethyl acetate/hexanes) afforded 5-(benzylthio)-2,3-difluoro-*N*,*N*-dimethylbenzamide (699 mg, 50% yield over five steps). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.28 (m, 5H), 7.28 – 7.20 (m, 1H), 7.13 (ddd, J_{F-H} = 10.0, 7.0 Hz, J_{H-H} = 2.3 Hz, 1H), 7.06 (ddd, J_{F-H} = 6.3, 1.1 Hz, J_{H-H} = 2.3 Hz, 1H), 4.11 (s, 2H), 3.13 (s, 3H), 2.89 (s, 3H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -135.39 (ddd, J_{F-F} = 22.0 Hz, J_{F-H} = 10.0, 1.1 Hz, 1F), -141.90 (ddd, J_{F-F} = 22.0 Hz, J_{F-H} = 7.0, 6.3 Hz, 1F).



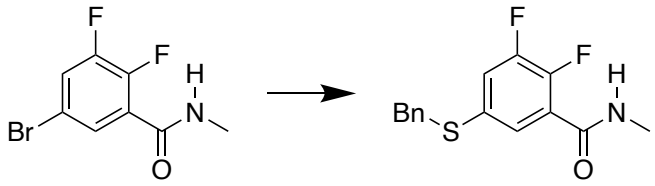
30 **31**

Synthesis of 31. To a solution of 5-(benzylthio)-2,3-difluoro-*N*,*N*-dimethylbenzamide (710 mg, 2.31 mmol, 1.0 eq) in acetonitrile (30 mL) was added acetic acid (0.5 mL, 9.25 mmol, 4 eq) and water (0.3 mL, 16.1 mmol, 7.0 eq). The mixture was cooled to 0 ˚C and 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione was added (1.073 g, 4.62 mmol, 2.0 eq). The ice bath was removed and the reaction mixture was stirred for one hour. Water was added and the mixture was extracted with ethyl acetate (x3). The organic extracts were washed with pH 7 buffer, water, brine, dried over Na_2SO_4, and concentrated. Purification of the crude oil by silica gel chromatography (1:4 ethyl acetate/hexanes) afforded 3-(dimethylcarbamoyl)-4,5-difluorobenzenesulfonyl chloride (291 mg, 45% yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 – 7.91 (m, 2H), 3.19 (s, 3H), 3.00 (s, 3H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -126.15 (dt, $J_{F\text{-}F}$ = 21.4 Hz, $J_{F\text{-}H}$ = 5.7 Hz 1F), -129.26 (ddd, $J_{F\text{-}F}$ = 21.4 Hz, $J_{F\text{-}H}$ = 9.0, 2.4 Hz, 1F).



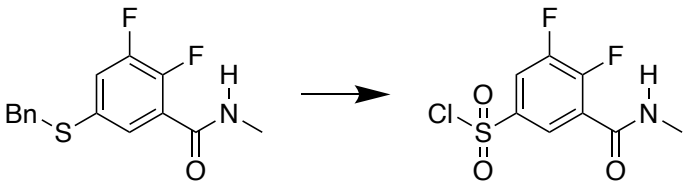
2 **32**

Synthesis of 32. To crude **2** (3.9 g, 16.4 mmol, 1.0 eq) was added dichloromethane (30 mL). To the solution was added oxalyl chloride (1.8 mL, 21.6 mmol, 1.3 eq), followed by 4 drops of DMF. The reaction was allowed to stir for 3 hours. The solvent was removed *in vacuo*. The resulting residue was dissolved in acetonitrile (30 mL). The residue was added to a thirty percent solution of methyl amine (8.2 mL) in THF dropwise at 0 °C. Water was added to the reaction mixture, and the mixture was extracted with ethyl acetate (x3). The combined organic washes were washed with water, brine, dried over Na_2SO_4, and concentrated. This gave crude 5-bromo-2,3-difluoro-*N*-methylbenzamide (3.42 g).



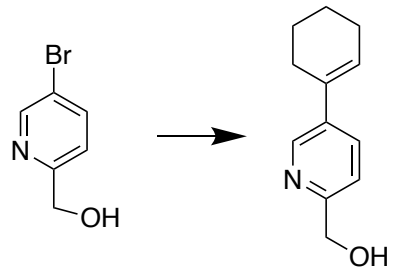
32 **33**

Synthesis of 33. To crude 5-bromo-2,3-difluoro-*N*-methylbenzamide (1.02 g, 4.0 mmol, 1.0 eq) was added Xantphos (250 mg, 0.4 mmol, 0.1 eq), followed by $Pd_2(dba)_3$ (198 mg, 0.2 mmol, 0.05 eq). The flask was evacuated and charged with argon (x3). The solids were dissolved in 1,4-dioxane (15 mL). To the resulting mixture was added DIPEA (1.5 mL, 8.6 mmol, 2.1 eq). Following the addition of the base, benzyl mercaptan (0.6 mL, 5.1 mmol, 1.2 eq) was added. The reaction mixture was heated at reflux for 16 hours. The mixture was allowed to cool to room temperature. Water was added, and the mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the crude solids by silica gel chromatography (15:85 ethyl acetate/hexanes) to afford 5-(benzylthio)-2,3-difluoro-*N*-methylbenzamide (390 mg, 33% yield over five steps). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (ddd, $J_{F\text{-}H}$ = 6.0, 1.8 Hz, $J_{H\text{-}H}$ = 2.5 Hz, 1H), 7.38 – 7.29 (m, 5H), 7.17 (ddd, $J_{F\text{-}H}$ = 9.8, 7.2 Hz, $J_{H\text{-}H}$ = 2.5 Hz, 1H), 6.58 (s, 1H), 4.15 (s, 2H), 3.05 (dd, J = 4.8, 1.1 Hz, 3H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -136.38 – -136.77 (m, 1F), -142.49 – -142.76 (m, 1F).



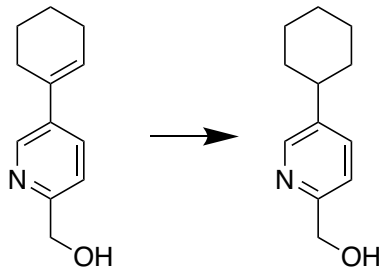
33 **34**

Synthesis of 34. To 5-(benzylthio)-2,3-difluoro-*N*-methylbenzamide (380 mg, 1.29 mmol, 1.0 eq) was added acetonitrile (5 mL). To the solution was added water (0.2 mL, 9.55 mmol, 7.4 eq), followed by acetic acid (0.3 mL, 5.44 mmol, 4.2 eq). The reaction mixture was cooled to 0 °C and 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione (632 mg, 2.72 mmol, 2.1 eq) was added. The reaction mixture was allowed to warm to room temperature and stir for an hour and a half. Water was added and the aqueous phase was extracted with ethyl acetate (x3). The combined organic extracts were washed with pH 7 buffer, water, brine, dried over sodium sulfate, and concentrated. Purification of the crude oil by silica gel chromatography (85:15 hexanes/ethyl acetate) gave 3,4-difluoro-5-(methylcarbamoyl)benzenesulfonyl chloride as a crystalline white solid (90 mg, 28% yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 – 7.95 (m, 2H), 3.54 (s, 3H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -95.98 – -96.29 (m, 1F), -109.70 (tt, J = 11.5, 8.1 Hz, 1F).



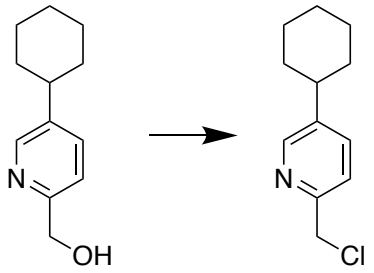
36 **37**

Synthesis of 37. To (5-bromopyridin-2-yl)methanol (5.0 g, 26.4 mmol, 1.0 eq) was added cyclohex-1-en-1-ylboronic acid (5.0 g, 39.6 mmol, 1.5 eq) followed by potassium phosphate tribasic (11.19 g, 52.8 mmol, 2.0 eq). The flask was evacuated and charged with argon (x3). The solids were dissolved in THF (50 mL) followed by the addition of degassed H_2O (1.0 mL, 52.8 mmol, 2.0 eq). Subsequently, Sphos (1.08 g, 2.6 mmol, 0.1 eq) and $Pd(OAc)_2$ (586 mg, 1.3 mmol, 0.05 eq) were added to the mixture. The reaction mixture was heated to 40 ˚C for 24 hours. The reaction mixture was allowed to cool to room temperature. Water was added and the mixture was extracted with EtOAc (x3 mL). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting oil via silica gel chromatography (4:6 EtOAc/hexanes) gave (5-(cyclohex-1-en-1-yl)pyridin-2-yl)methanol as a clear oil (4.49 g, 90% yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 (d, J = 2.2 Hz, 1H), 7.67 (dd, J = 8.2, 2.2 Hz, 1H), 7.19 (d, J = 8.2 Hz, 1H), 6.18 (m, 1H), 4.76 (s, 2H), 2.41 (m, 2H), 2.25 (m, 2H), 1.82 (m, 2H), 1.70 (m, 2H).



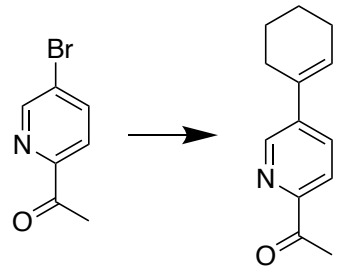
37 **38**

Synthesis of 38. To (5-(cyclohex-1-en-1-yl)pyridin-2-yl)methanol (4.463 g, 23.7 mmol, 1.0 eq) was added PtO_2 (446 mg, 1.96 mmol, 0.082 eq). The round bottom was evacuated and charged with argon (x3). The round bottom flask was evacuated and charged with H_2 (x3). Ethyl acetate (25 mL) and methanol (25 mL) were added to the flask. The reaction mixture was allowed to stir at room temperature for 24 hours. The reaction was monitored for completeness by use of ^1H NMR. Ethyl acetate was added to the reaction mixture, and it was filtered over Celite. The Celite was washed (x3) with EtOAc and the filtrate was concentrated. This afforded (5-cyclohexylpyridin-2-yl)methanol (4.26 g, 96% yield) as a yellow oil. Spectroscopic Data: ^1H NMR (300 MHz, $CDCl_3$) δ 8.40 (d, J = 2.2 Hz, 1H), 7.53 (dd, J = 8.0, 2.2 Hz, 1H), 7.20 (d, J = 8.0 Hz, 1H), 4.73 (s, 2H), 2.53 (ddt, J = 11.6, 5.1, 2.5 Hz, 1H), 1.93 – 1.74 (m, 5H), 1.53 – 1.30 (m, 5H).



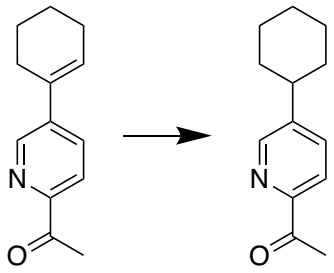
38 **39**

Synthesis of 39. To (5-cyclohexylpyridin-2-yl)methanol (4.259 g, 22.4 mmol, 1.0 eq) was added DCM (80 mL) followed by thionyl chloride (2.4 mL, 30.9 mmol, 1.4 eq). The reaction mixture was allowed to stir at room temperature for 3 hours. The solvent was removed under vacuum to give 2-(chloromethyl)-5-cyclohexylpyridine in quantitative yield. The product was not characterized.



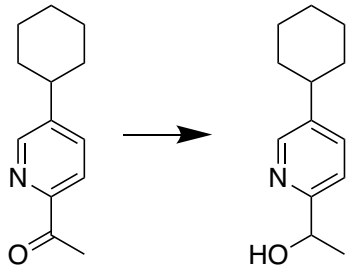
40 **41**

Synthesis of 41. To 1-(5-bromopyridin-2-yl)ethan-1-one (650 mg, 3.25 mmol, 1.0 eq) was added cyclohex-1-en-1-ylboronic acid (756 mg, 6.0 mmol, 1.8 eq) followed by potassium phosphate tribasic (1.272 g, 6.0 mmol, 1.8 eq). The solids were dissolved in THF (20 mL) followed by the addition of degassed H_2O (0.117 mL, 6.5 mmol, 2.0 eq), Sphos (123 mg, 0.3 mmol, 0.09 eq), and $Pd(OAc)_2$ (33 mg, 0.15 mmol, 0.05 eq). The resulting mixture was heated to 40 ˚C for twenty four hours. The reaction mixture was allowed to cool to room temperature. Water was added and the mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the crude oil via silica gel chromatography (2:8 ethyl acetate/hexanes) afforded 1-(5-(cyclohex-1-en-1-yl)pyridin-2-yl)ethan-1-one (380 mg, 58% yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.75 (d, J = 2.3 Hz, 1H), 8.04 (d, J = 8.3 Hz, 1H), 7.86 (dd, J = 8.3, 2.3 Hz, 1H), 6.39 (tt, J = 4.1, 1.7 Hz, 1H), 2.82 – 2.77 (m, 3H), 2.44 (m, 2H), 2.34 – 2.26 (m, 2H), 1.89 – 1.80 (m, 2H), 1.77 – 1.66 (m, 2H).



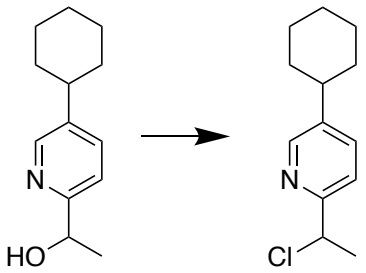
41 **42**

Synthesis of 42. To 1-(5-(cyclohex-1-en-1-yl)pyridin-2-yl)ethan-1-one (380 mg, 1.89 mmol, 1.0 eq) was added PtO_2 (40 mg, 0.2 mmol, 0.1 eq). The round bottom was evacuated and charged with H_2 (x3). Then, EtOAc (10 mL) and methanol (10 mL) were added. The reaction mixture was allowed to stir at room temperature for twenty-four hours. Ethyl acetate was added and it was filtered over Celite. The Celite was washed with ethyl acetate (x3) and the filtrate was concentrated under reduced pressure. This gave 1-(5-cyclohexylpyridin-2-yl)ethan-1-one quantitatively. Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 (d, J = 2.1 Hz, 1H), 7.92 (d, J = 8.0 Hz, 1H), 7.71 (dd, J = 8.0, 2.1 Hz, 1H), 7.44 – 7.32 (m, 1H), 2.75 – 2.55 (m, 1H), 2.05 – 1.11 (m, 13H).



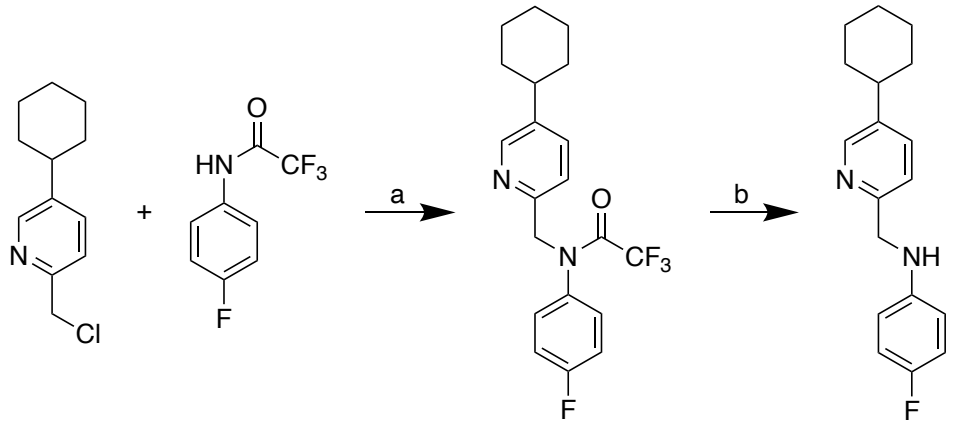
42 **43**

Synthesis of 43. To 1-(5-cyclohexylpyridin-2-yl)ethan-1-one (390 mg, 1.9 mmol, 1.0 eq) was added MeOH (10 mL) followed by $NaBH_4$ (363 mg, 9.5 mmol, 5.0 eq) at 0 ˚C. The reaction mixture was allowed to warm to room temperature and stirred for three hours. Water and the reaction mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the crude solid by silica gel chromatography (4:6 ethyl acetate/hexanes) gave 1-(5-cyclohexylpyridin-2-yl)ethan-1-ol (366 mg, 93% yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.82 (d, J = 8.2 Hz, 1H), 7.46 (d, J = 8.2 Hz, 1H), 5.11 (q, J = 6.7 Hz, 1H), 2.63 (m, 1H), 1.97 – 1.76 (m, 6H), 1.63 – 1.56 (d, J = 6.7 Hz, 3H), 1.51 – 1.38 (m, 4H).



43 **44**

Synthesis of 44. To 1-(5-cyclohexylpyridin-2-yl)ethan-1-ol (358 mg, 1.75 mmol, 1.0 eq) was added DCM (10 mL) followed by thionyl chloride (0.15 mL, 2.0 mmol, 1.15 eq). The reaction mixture was allowed to stir at room temperature for three hours. Dichloromethane was added and the reaction mixture was washed with aqueous sodium bicarbonate. The organic phase was washed with water, brine, and dried over Na_2SO_4. The organic phase was concentrated under vacuum to give 2-(1-chloroethyl)-5-cyclohexylpyridine (quantitative yield) which was stored as a 1M solution in toluene under Ar. No data was collected due to the instability of the product. No purification was performed before it was used in the next step.



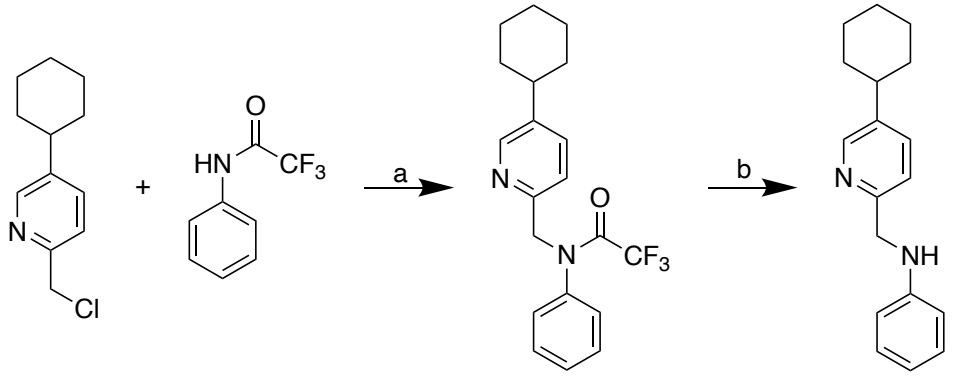
39 **45** **46** **47**

Synthesis of 47.

a) To 2,2,2-trifluoro-*N*-(4-fluorophenyl)acetamide **45** (500 mg, 2.4 mmol, 1.0 eq) was added NaI (72 mg, 0.48 mmol, 0.2 eq) and Cs_2CO_3 (3.67 g, 11.2 mmol, 4.6 eq). The solids were dissolved in acetonitrile (20 mL). A 1M solution of (chloromethyl)-5-cyclohexylpyridine **39** (3.1 mL, 3.1 mmol, 1.3 eq) in toluene was added to the reaction mixture which was heated to 60 ˚C and then allowed to stir for 16 hours. The reaction mixture was allowed to cool to room temperature. Saturated ammonium chloride solution was added, and the mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. This gave crude *N*-((5-cyclohexylpyridin-2-yl)methyl)-2,2,2-trifluoro-*N*-(4-fluorophenyl)acetamide **46** (508 mg).

b) To crude *N*-((5-cyclohexylpyridin-2-yl)methyl)-2,2,2-trifluoro-*N*-(4-fluorophenyl)acetamide **46** (508 mg, 1.33 mmol, 1.0 eq) was added K_2CO_3 (544 mg, 3.9 mmol, 3.0 eq) followed by THF (10 mL) and methanol (10 mL). The resulting mixture was allowed to stir at room temperature for four hours. Saturated ammonium chloride solution was added. The mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the crude solid by silica gel chromatography (2:8 ethyl acetate/hexanes) afforded *N*-((5-cyclohexylpyridin-2-yl)methyl)-4-fluoroaniline **47** (443 mg, 65% yield over two steps) as a light green oil. Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.46 (d, $J_{H\text{-}H}$ = 2.2 Hz, 1H), 7.65 (dd, $J_{H\text{-}H}$ = 8.1, 2.2 Hz, 1H), 7.38 (d, $J_{H\text{-}H}$ = 8.1 Hz, 1H), 6.89

(dd, J_{F-H} = 8.6 Hz, J_{H-H} = 9.0 Hz, 2H), 6.62 (dd, J_{F-H} = 4.2 Hz, J_{H-H} = 9.0 Hz, 2H), 4.49 (s, 2H), 2.58 (m, 1H), 1.84 (m, 5H), 1.57 – 1.18 (m, 5H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -127.62 (dd, J_{F-H} = 8.6, 4.2 Hz, 1F). HRMS (ESI) m/z = 307.16888 [M+Na]+, HRMS (ESI+) calculated for $C_{18}H_{21}FN_2$: 284.1688, found 284.1686.



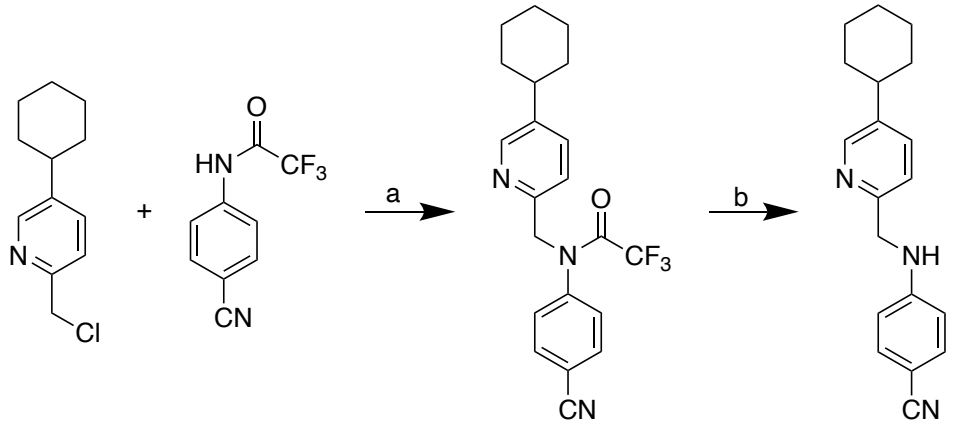
39 **52** **53** **54**

Synthesis of 54.

a) To 2,2,2-trifluoro-*N*-phenylacetamide **52** (460 mg, 2.43 mmol, 1.0 eq) was added NaI (99 mg, 0.66, 0.2 eq) and Cs_2CO_3 (3.5 g, 10.7 mmol, 4.4 eq). The solids were dissolved in acetonitrile (30 mL). A 1M solution of (chloromethyl)-5-cyclohexylpyridine **39** (3.4 mL, 3.4 mmol, 1.4 eq) in toluene was added to the reaction mixture which was heated to 60 ˚C and then allowed to stir for 16 hours. The reaction mixture was allowed to cool to room temperature. Saturated ammonium chloride solution was added, and the mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, and dried over Na_2SO_4. Filtration through a pad of silica gave crude *N*-((5-cyclohexylpyridin-2-yl)methyl)-2,2,2-trifluoro-*N*-phenylacetamide **53** (420 mg).

b) To crude *N*-((5-cyclohexylpyridin-2-yl)methyl)-2,2,2-trifluoro-*N*-phenylacetamide **53** (420 mg, 1.16 mmol, 1.0 eq) was added K_2CO_3 (326 mg, 2.3 mmol, 2.0 eq) followed by THF (8 mL) and methanol (8 mL). The resulting mixture was allowed to stir at room temperature for four hours. Saturated ammonium chloride solution was added. The mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the crude solid by silica gel chromatography (2:8 ethyl acetate/ hexanes) afforded *N*-((5-cyclohexylpyridin-2-yl)methyl)aniline **54** (333 mg, 53% yield over two steps) as a green oil. Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45 (d, J = 2.3 Hz, 1H),

7.50 (dd, J = 8.0, 2.3 Hz, 1H), 7.28 (d, J = 8.0 Hz, 1H), 7.20 (td, J = 7.4, 1 Hz, 2H), 6.78 – 6.66 (m, 3H), 4.75 (s, 1H), 4.44 (d, J = 4.7 Hz, 2H), 2.65 – 2.45 (m, 1H), 1.96 – 1.66 (m, 5H), 1.55 – 1.18 (m, 5H); HRMS (ESI) m/z = 289.1689 [M+Na]+, HRMS (ESI+) calculated for $C_{18}H_{22}N_2$: 266.1783, found 266.1789.



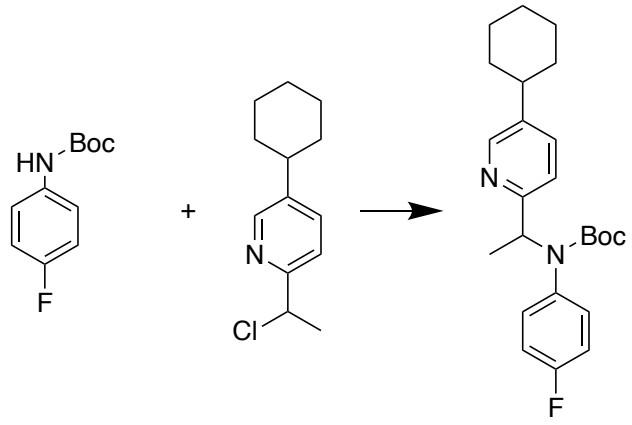
39 **58** **59** **60**

Synthesis of 60.

a) To *N*-(4-cyanophenyl)-2,2,2-trifluoroacetamide **58** (800 mg, 3.73 mmol, 1.0 eq) was added NaI (112 mg, 0.74 mmol, 0.2 eq) and K_2CO_3 (1.03 g, 7.45 mmol, 2.0 eq). The solids were dissolved in acetonitrile (25 mL). A 1M solution of (chloromethyl)-5-cyclohexylpyridine **39** (4.8 mL, 4.8 mmol, 1.3 eq) in toluene was added to the reaction mixture which was heated to 60 ˚C and then allowed to stir for 16 hours. The reaction mixture was allowed to cool to room temperature. Saturated ammonium chloride solution was added, and the mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. This afforded *N*-(4-cyanophenyl)-*N*-((5-cyclohexylpyridin-2-yl)methyl)-2,2,2-trifluoroacetamide **59** (1.002 g) as an impure mixture.

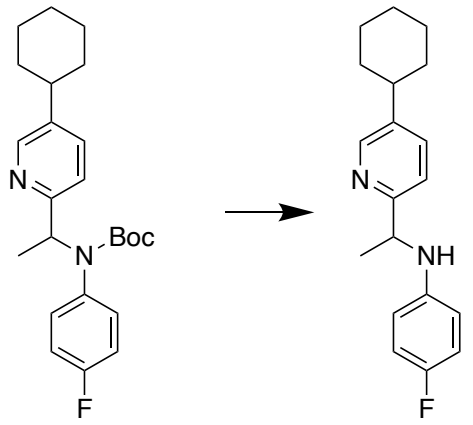
b) To crude *N*-(4-cyanophenyl)-*N*-((5-cyclohexylpyridin-2-yl)methyl)-2,2,2-trifluoroacetamide **59** (1.002 g, 2.58 mmol, 1.0 eq) was added K_2CO_3 (714 mg, 5.16 mmol, 2.0 eq) followed by THF (25 mL) and methanol (25 mL). The resulting mixture was allowed to stir at room temperature for four hours. Saturated ammonium chloride solution was added. The reaction mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the crude solid by silica gel chromatography (2:8 ethyl acetate/hexanes) gave 4-(((5-cyclohexylpyridin-2-yl)methyl)amino)benzonitrile **60** (374 mg, 35% yield over two steps). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45 (d, *J* = 2.0 Hz, 1H), 7.53 (dd, *J* = 7.9, 2.3 Hz, 1H), 7.46

(d, *J* = 8.7 Hz, 2H), 7.22 (d, *J* = 7.9 Hz, 1H), 6.66 (d, *J* = 8.7 Hz, 2H), 5.52 (t, 5.0 Hz, 1H), 4.45 (d, *J* = 5.0 Hz, 2H), 2.65 – 2.48 (m, 1H), 1.92 – 1.76 (m, 5H), 1.54 – 1.22 (m, 5H).



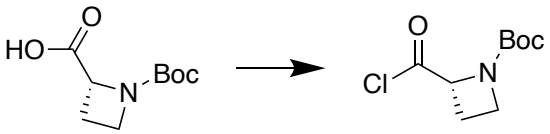
70 **44** **71**

Synthesis of 71. To *tert*-butyl (4-fluorophenyl)carbamate **70** (277 mg, 0.71 mmol, 1.0 eq) was added NaI (40 mg, 0.2 mmol, 0.25 eq) and Cs_2CO_3 (1.943 g, 6.15 mmol, 8.4 eq). The solids were dissolved in acetonitrile (13 mL). A 1M solution of 2-(1-chloroethyl)-5-cyclohexylpyridine **44** (1.7 mL, 1.724 mmol, 2.0 eq) in toluene was added to the reaction mixture which was heated to 60 ˚C and allowed to stir for 16 hours. The reaction was allowed to cool to room temperature. Saturated ammonium chloride solution was added, and the mixture was extracted with ethyl acetate (x3). The combined organic extracts were then washed with water, brine, and dried over Na_2SO_4, and concentrated. Purification of the crude oil by column chromatography (2:8 ethyl acetate/hexanes) afforded *tert*-butyl (1-(5-cyclohexylpyridin-2-yl)ethyl)(4-fluorophenyl)carbamate **71** (449 mg, 88% yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.44 (d, $J_{H\text{-}H}$ = 1.9 Hz, 1H), 7.61 (dd, $J_{H\text{-}H}$ = 8.2, 1.9 Hz, 1H), 7.46 (d, $J_{H\text{-}H}$ = 8.2 1H), 7.31 (dd, 7.8, $J_{F\text{-}H}$ = 4.7 Hz, $J_{H\text{-}H}$ = 7.8 Hz, 2H), 7.00 (dd, $J_{F\text{-}H}$ = 8.5 Hz, $J_{H\text{-}H}$ = 7.8 2H), 5.21 (q, 7.2 Hz, 1H), 2.57 (m, 1H), 1.94 – 1.71 (m, 8H), 1.55 (s, 9H), 1.48 – 1.32 (m, 5H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -120.16 (dd, $J_{F\text{-}H}$ = 8.5, 4.7 Hz, 1F).



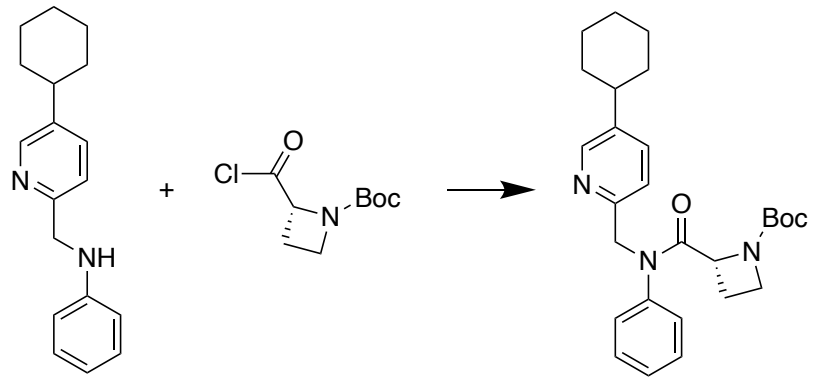
71 **65**

Synthesis of 65. To *tert*-butyl (1-(5-cyclohexylpyridin-2-yl)ethyl)(4-fluorophenyl)carbamate **71** (60 mg, 0.15 mmol, 1.0 eq) in DCM (10 mL) was added TFA (0.6 mL, 7.84 mmol, 52.0 eq). The reaction was allowed to stir at room temperature for one hour. The reaction mixture was quenched with aqueous sodium bicarbonate and was extracted with dichloromethane (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentration. Purification of the crude oil by silica gel chromatography (2:8 ethyl acetate/hexanes) afforded *N*-(1-(5-cyclohexylpyridin-2-yl)ethyl)-4-fluoroaniline **65** (25 mg, 57% yield) which was used directly in the next reaction.



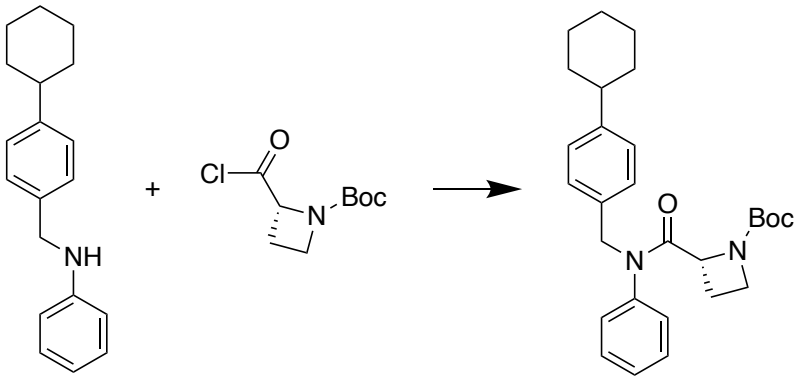
72 **48**

Synthesis of 48. *Tert*-butyl (*R*)-2-(chlorocarbonyl)azetidine-1-carboxylate **72** (186 mg, 0.925 mmol, 1.0 eq) was dissolved in dichloromethane (10 mL). Subsequently, oxalyl chloride (0.1 mL, 1.30 mmol, 1.4 eq) followed by two drops of DMF was added to the reaction mixture. The reaction mixture was allowed to stir for three hours. The reaction solvent was removed *in vacuo*. Due to product instability it was not characterized, and was used in the subsequent reaction immediately following concentration.



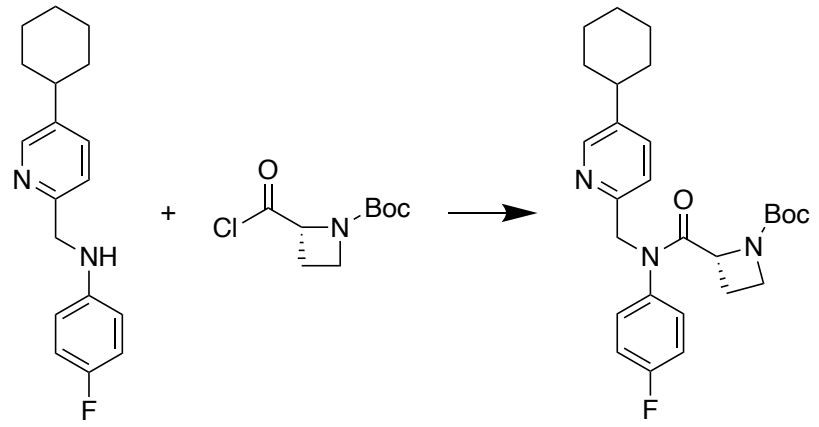
54 **48** **55**

Synthesis of 55. To a stirred solution of *N*-((5-cyclohexylpyridin-2-yl)methyl)aniline **54** (651 mg, 2.44 mmol, 1.0 eq) in THF (15 mL) at 0 ˚C was added a solution of 1.4M MeMgBr (3.1 mL, 4.354 mmol, 1.8 eq) in 1:3 toluene/tetrahydrofuran. The reaction mixture was allowed to stir for fifteen minutes before *tert*-butyl (*R*)-2-(chlorocarbonyl)azetidine-1-carboxylate **48** (1.068 g, 4.88 mmol, 2.0 eq) in THF (10 mL) was added. The ice bath was removed and the reaction mixture was allowed to reach room temperature. After two and a half hours saturated ammonium chloride solution was added. The mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the crude oil by silica gel chromatography (4:6 ethyl acetate/hexanes) afforded *tert*-butyl (*R*)-2-(((5-cyclohexylpyridin-2-yl)methyl)(phenyl)carbamoyl)azetidine-1-carboxylate **55** (312 mg, 43% yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (d, *J* = 2.2 Hz, 1H), 7.42 – 7.29 (m, 3H), 7.28 – 7.23 (m, 1H), 7.06 – 6.93 (m, 2H), 6.82 (d, *J* = 8.1 Hz, 1H), 4.82 (m, 3H), 3.93 (dq, *J* = 13.8, 8.0 Hz, 2H), 2.55 – 2.40 (m, 2H), 1.83 (m, 6H), 1.48-1.2 (m, 14H). HRMS (ESI) m/z = 450.2746 [M+H]+, HRMS (ESI+) calculated for $C_{27}H_{35}N_3O_3$: 449.2678, found 449.2672.



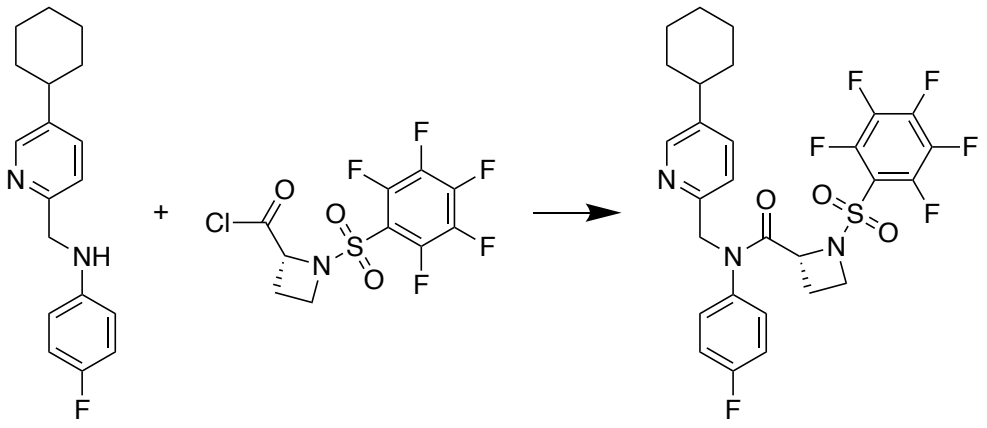
67 **48** **68**

Synthesis of 68. To a stirred solution of *N*-(4-cyclohexylbenzyl)aniline **67** (255 mg, 0.96 mmol, 1.0 eq) in THF (7 mL) at 0 ˚C was added a solution of 1.4M MeMgBr (1.7 mL, 2.38 mmol, 2.5 eq) in 1:3 toluene/tetrahydrofuran. The reaction mixture was allowed to stir for fifteen minutes before *tert*-butyl (*R*)-2-(chlorocarbonyl)azetidine-1-carboxylate **48** (420 mg, 1.92 mmol, 2.0 eq) in THF (7 mL) was added. The ice bath was removed and the reaction mixture was allowed to reach room temperature. After two and a half hours saturated ammonium chloride solution was added. The reaction mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting oil by silica gel chromatography (4:6 ethyl acetate/hexanes) afforded *tert*-butyl (*R*)-2-((4-cyclohexylbenzyl)(phenyl)carbamoyl)azetidine-1-carboxylate **68** (108 mg, 25% yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.29 (m, 3H), 7.19 – 7.07 (m, 4H), 7.05 – 6.90 (m, 2H), 5.05 (s, 1H), 4.63 (s, 1H), 4.55 – 4.41 (m, 1H), 4.07 – 3.81 (m, 1H), 3.73 (td, *J* = 8.3, 5.7 Hz, 1H), 2.55 – 2.37 (m, 1H), 2.21 – 2.00 (m, 2H), 1.89 – 1.70 (m, 5H), 1.48 – 1.32 (m, 14H); HRMS (ESI) m/z = 471.2611 [M+Na]+, HRMS (ESI+) calculated for $C_{28}H_{36}N_2O_3$: 4489.2725, found 448.2718.



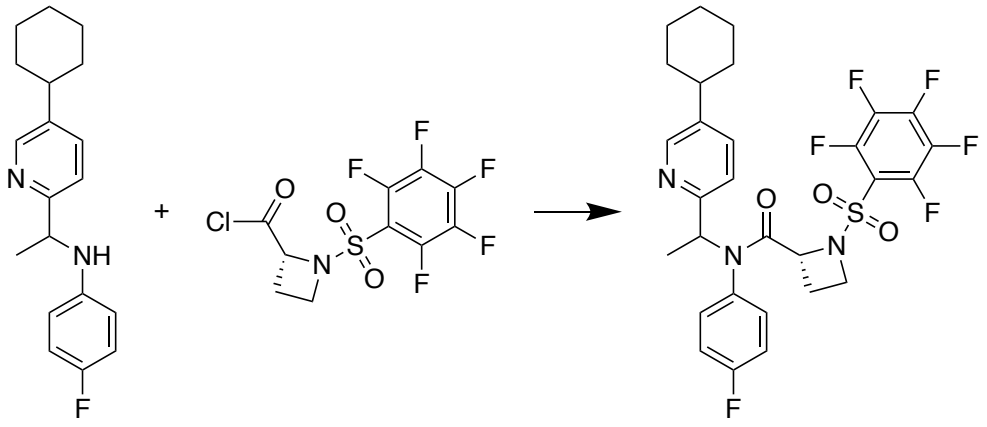
47 **48** **49**

Synthesis of 49. To a stirred solution of *N*-((5-cyclohexylpyridin-2-yl)methyl)-4-fluoroaniline **47** (223 mg, 0.785 mmol, 1.0 eq) in THF (6 mL) at 0 °C was added a solution of 1.4M MeMgBr (1.4 mL, 1.0 mmol, 1.3 eq) in 1:3 toluene/tetrahydrofuran. The reaction mixture was allowed to stir for fifteen minutes before *tert*-butyl (*R*)-2-(chlorocarbonyl)azetidine-1-carboxylate **48** (343 mg, 1.56, 2.0 eq) in THF (6 mL) was added. The ice bath was removed and the reaction was allowed to reach room temperature. After two and a half hours saturated ammonium chloride solution was added. The mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting oil by silica gel chromatography (4:6 ethyl acetate/hexanes) gave *tert*-butyl (*R*)-2-(((5-cyclohexylpyridin-2-yl)methyl)(4-fluorophenyl)carbamoyl)azetidine-1-carboxylate **49** (118 mg, 33% yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (d, J_{H-H} = 2.3 Hz, 1H), 7.50 (dd, J_{H-H} = 8.2, 2.3 Hz, 1H), 7.42 (d, J_{H-H} = 8.2 Hz, 1H), 7.26 – 7.08 (m, 2H), 7.02 (t, J_{F-H} = 8.5 Hz, 2H), 5.08 – 4.93 (m, 1H), 4.54 (m, 1H), 4.16 – 3.98 (m, 1H), 3.74 (td, J_{H-H} = 8.1, 5.8 Hz, 1H), 3.67 – 3.52 (m, 1H), 2.59 – 2.43 (m, 1H), 2.22 – 2.07 (m, 1H), 1.93 – 1.69 (m, 6H), 1.57 – 1.13 (m, 14H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -112.92 (m, 1F). HRMS (ESI) m/z = 490.2467 [M+Na]+, HRMS (ESI+) calculated for $C_{27}H_{34}FN_3O_3$: 467.2584, found 467.2580.



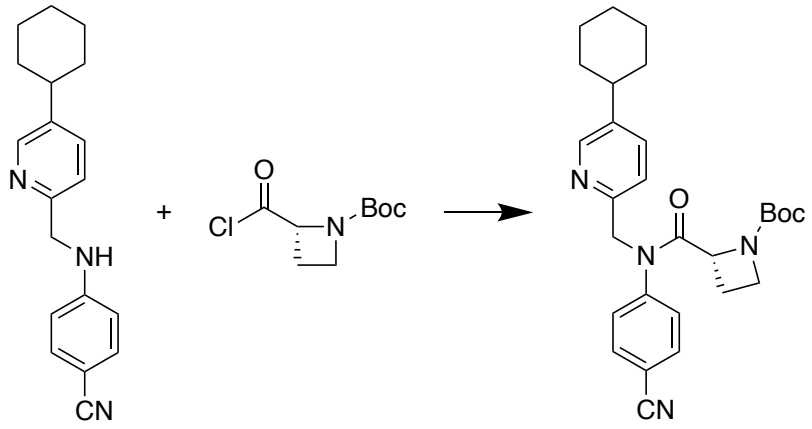
47 **66** **S3I-H247**

Synthesis of S3I-H247. To a solution of *N*-(1-(5-cyclohexylpyridin-2-yl)ethyl)-4-fluoroaniline **47** (88 mg, 0.30 mmol, 1.0 eq) in THF (5 mL) at 0 ˚C was added a solution of 1.4 M MeMgBr (0.55 mL, 0.779 mmol, 2.6 eq) in 1:3 toluene/tetrahydrofuran. The reaction mixture was allowed to stir for fifteen minutes. (*R*)-1-((perfluorophenyl)sulfonyl)azetidine-2-carbonyl chloride **66** (141 mg, 0.6 mmol, 2.0 eq) in THF (5 mL) was added to the reaction mixture. The ice-bath was removed and the reaction mixture was allowed to warm to room temperature. After two and a half hours saturated ammonium chloride solution was added. The reaction was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting oil by silica gel chromatography (1:1 ethyl acetate/hexanes) afforded (2*R*)-*N*-(1-(5-cyclohexylpyridin-2-yl)ethyl)-*N*-(4-fluorophenyl)-1-((perfluorophenyl)sulfonyl)azetidine-2-carboxamide **S3I-H247** (36 mg, 20% yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, $J_{H\text{-}H}$ = 2.3 Hz, 1H), 7.57 (dd, $J_{H\text{-}H}$ = 8.0, 2.3 Hz, 1H), 7.26 (d, $J_{H\text{-}H}$ = 8.0 Hz, 1H), 7.16 (dd, $J_{F\text{-}H}$ = 5.0 Hz, $J_{H\text{-}H}$ = 9.0 Hz, 2H), 7.06 (dd, $J_{F\text{-}H}$ = 7.9 Hz, $J_{H\text{-}H}$ = 9.0 Hz, 2H), 5.14 – 4.79 (m, 3H), 4.20 – 4.01 (m, 2H), 2.65 – 2.45 (m, 1H), 2.32 (ddt, $J_{H\text{-}H}$ = 10.7, 9.0, 7.0 Hz, 1H), 2.06 – 1.75 (m, 5H), 1.49 – 1.29 (m, 5H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -111.44 (dd, $J_{F\text{-}H}$ = 7.9, 5.0 Hz, 1F), -135.92 (dt, $J_{F\text{-}F}$ = 21.4, 5.5 Hz, 2F), -146.85 (t, $J_{F\text{-}F}$ = 20.9 Hz, 1F), -159.56 (tt, $J_{F\text{-}F}$ = 20.9, 6.0 Hz, 2F); HPLC purity = 100%; HRMS (ESI) m/z 620.1393 $[M + Na]^+$, HRMS (ESI+) calculated for $C_{28}H_{25}F_6N_3O_3S$: 597.1520, found 597.1504; EMSA IC_{50} 4-5 μM.



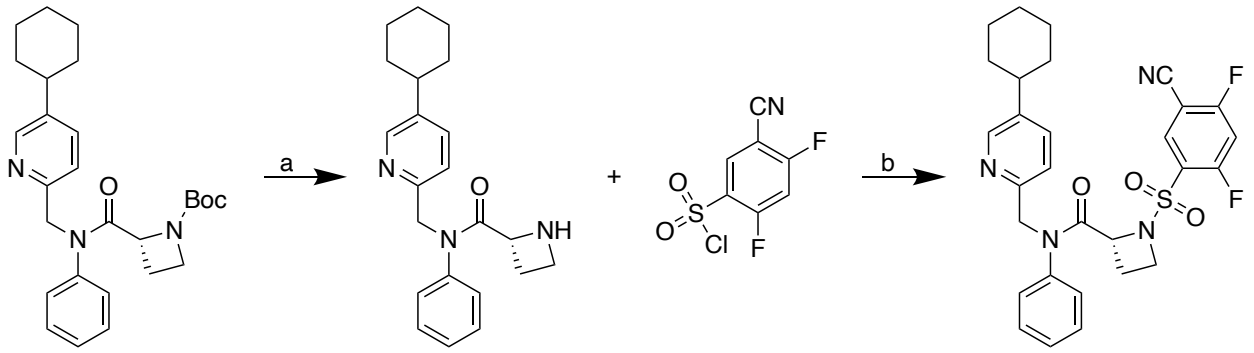
65 **66** **S3I-H287**

Synthesis of S3I-H287. To a solution of *N*-(1-(5-cyclohexylpyridin-2-yl)ethyl)-4-fluoroaniline **65** (25 mg, 83.0 μmol, 1.0 eq) in THF (3 mL) at 0 ˚C was added a solution of 1.4 M MeMgBr (80 μl, 112.0 μmol, 1.4 eq) in 1:3 toluene/tetrahydrofuran. The reaction mixture was allowed to stir for fifteen minutes. (*R*)-1-((perfluorophenyl)sulfonyl)azetidine-2-carbonyl chloride **66** (58 mg, 0.16 mmol, 2.0 eq) in THF (3 mL) was added to the reaction mixture. The ice-bath was removed and the reaction was allowed to warm to room temperature. After two and a half hours saturated ammonium chloride solution was added. The mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting oil by silica gel chromatography (1:1 ethyl acetate/ hexanes) followed by purification by HPLC 1.8 mL/min flow rate 80:20 acetonitrile/water on a Phenomenal column Luna 5u PFP 100A 250 x 10 mm with a PDA detector afforded (2*R*)-*N*-(1-(5-cyclohexylpyridin-2-yl)ethyl)-*N*-(4-fluorophenyl)-1-((perfluorophenyl)sulfonyl)azetidine-2-carboxamide **S3I-H287** (3 mg, 6% yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (d, $J_{H\text{-}H}$ = 2.3 Hz, 1H), 7.52 (dd, $J_{H\text{-}H}$ = 8.1, 2.3 Hz, 1H), 7.34 – 6.54 (m, 4H), 5.91 (p, $J_{H\text{-}H}$ = 7.0 Hz, 1H), 4.73 (dd, $J_{H\text{-}H}$ = 9.1, 7.0 Hz, 1H), 4.13 (dt, $J_{H\text{-}H}$ = 8.9, 7.1 Hz, 1H), 4.08 – 3.98 (m, 1H), 2.53 (m, 1H), 2.28 (ddt, $J_{H\text{-}H}$ = 10.9, 9.1, 7.1 Hz, 1H), 2.02 – 1.63 (m, 6H), 1.55 – 1.12 (m, 8H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -111.50 (dd, $J_{F\text{-}H}$ = 7.0, 6.4 Hz, 1F), -135.72 – -135.95 (m, 2F), -147.03 (tt, $J_{F\text{-}F}$ = 21.3, 6.4 Hz, 1F), -159.56 – -159.85 (m, 2F); HPLC purity = 100%; HRMS (ESI) m/z 634.1580 $[M + Na]^+$, HRMS (ESI+) calculated for $C_{28}H_{25}F_6N_3O_3S$: 611.1677, found 611.1684; EMSA IC_{50} 2.8 μM.



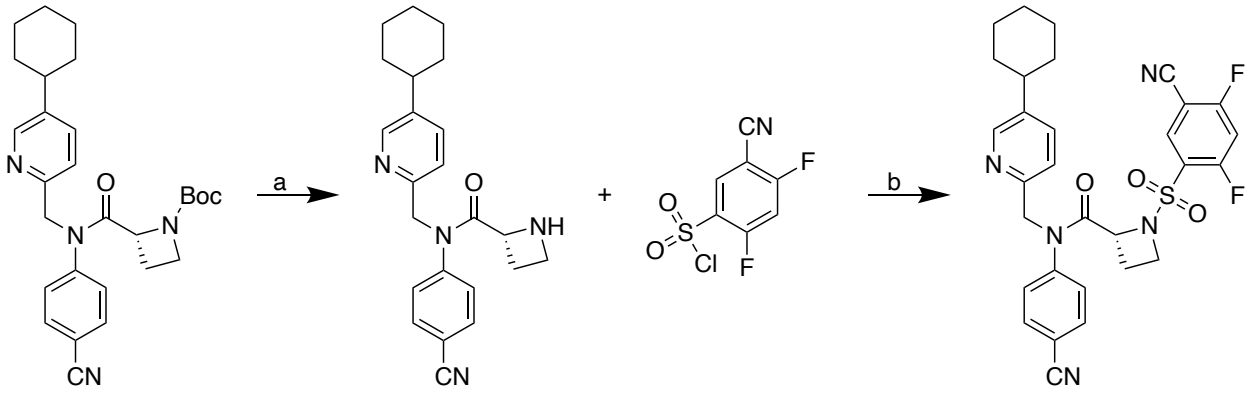
60 **48** **61**

Synthesis of 61. To a stirred solution of 4-(((5-cyclohexylpyridin-2-yl)methyl)amino)benzonitrile **60** (130 mg, 0.44 mmol, 1.0 eq) in THF (5.0 mL) at 0 °C was added a solution of 1.0 M MeMgBr (1.15 mL, 1.15 mmol, 2.6 eq) in 1:3 toluene/tetrahydrofuran. The reaction mixture was allowed to stir for fifteen minutes before *tert*-butyl (*R*)-2-(chlorocarbonyl)azetidine-1-carboxylate **48** (202 mg, 0.92 mmol, 2.1 eq) in THF (5 mL) was added. The ice bath was removed and the reaction mixture was allowed to reach room temperature. After two and a half hours saturated ammonium chloride solution was added. The mixture was extracted with ethyl acetate (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting oil by silica gel chromatography (4:6 ethyl acetate/hexanes) afforded *tert*-butyl (*R*)-2-((4-cyanophenyl)((5-cyclohexylpyridin-2-yl)methyl)carbamoyl)azetidine-1-carboxylate **61** (108 mg, 58 % yield). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, J = 2.3 1H), 7.68 (d, J = 8.1 Hz, 2H), 7.51 (dd, J = 8.1, 2.3 Hz, 1H), 7.41 (m, 3H), 5.04 (s, 2H), 4.60 (m, 1H), 4.10 (q, J = 7.7 Hz, 1H), 3.79 (q, J = 7.7 Hz, 1H), 2.52 (m, 1H), 2.27 – 2.10 (m, 2H), 1.82 (m, 5H), 1.54 – 1.25 (m, 14H).

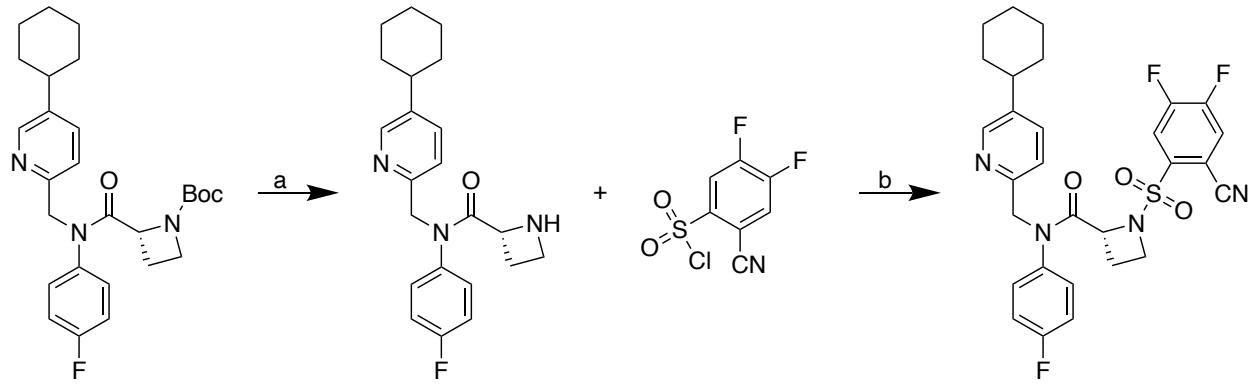


55 **56** **23** **S3I-H289**

Synthesis of S3I-H289. To a solution of *tert*-butyl (*R*)-2-(((5-cyclohexylpyridin-2-yl)methyl)(phenyl)carbamoyl)azetidine-1-carboxylate **55** (85 mg, 0.18 mmol, 1.0 eq) in DCM (10 mL) was added TFA (0.85 mL, 11.1 mmol, 62.0 eq). The reaction mixture was stirred at room temperature for one hour. The reaction mixture was concentrated and the resulting oil was used directly in the next reaction. The oil was dissolved in DCM (5 mL), and DIPEA (0.19 mL, 1.1 mmol, 6.0 eq) was added to the solution at 0 ˚C. The reaction mixture was allowed to stir for fifteen minutes. 5-cyano-2,4-difluorobenzenesulfonyl chloride **23** (80 mg, 0.33 mmol, 1.8 eq) in DCM (5 mL) was added. The ice bath was removed and the reaction was allowed to warm to room temperature. After two and a half hours saturated ammonium chloride solution was added. The mixture was extracted with dichloromethane (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting oil by silica gel chromatography (1:1 ethyl acetate/hexanes) afforded (*R*)-1-((5-cyano-2,4-difluorophenyl)sulfonyl)-*N*-((5-cyclohexylpyridin-2-yl)methyl)-*N*-phenylazetidine-2-carboxamide **S3I-H289** (42 mg, 41% yield over two steps). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, J_{H-H} = 2.3 Hz, 1H), 8.25 (t, J_{H-H} = 7.1 Hz, 1H), 7.51 (dd, J_{H-H} = 8.1, 2.3 Hz, 1H), 7.38 (m, 3H), 7.24 – 7.04 (m, 4H), 5.05 – 4.80 (m, 3H), 4.18 – 4.06 (m, 1H), 3.94 (td, J_{H-H} = 8.1, 4.6 Hz, 1H), 2.63 – 2.45 (m, 1H), 2.42 – 2.27 (m, 1H), 1.96 – 1.67 (m, 6H), 1.52 – 1.29 (m, 5H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -92.58 (dt, J_{F-F} = 16.6 Hz, J_{F-H} = 8.2 Hz, 1F), -94.7 (ddd, J_{F-F} = 16.6 Hz, J_{F-H} = 8.7, 7.1 Hz, 1F); HPLC purity = 100%; HRMS (ESI) m/z = 573.1752 $[M + Na]^+$, HRMS (ESI+) calculated for $C_{29}H_{28}F_2N_4O_3S$: 550.1850, found 550.1858; EMSA IC_{50} 4-5 μM.



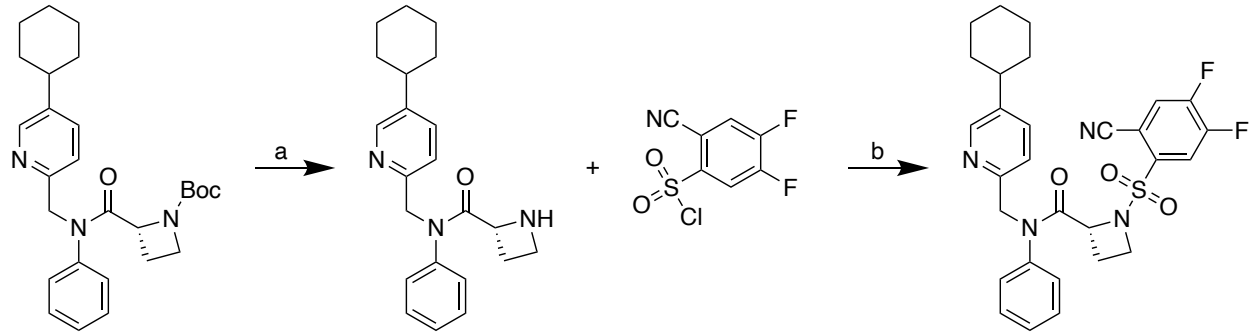
Synthesis of S3I-H275. To a stirred solution of *tert*-butyl (*R*)-2-((4-cyanophenyl)((5-cyclohexylpyridin-2-yl)methyl)carbamoyl)azetidine-1-carboxylate **61** (65 mg, 0.13 mmol, 1.0 eq) in DCM (10 mL) was added TFA (0.7 mL, 8.49 mmol, 65.0 eq). The reaction mixture was stirred at room temperature for one hour. The reaction was concentrated and the resulting oil was used directly in the next reaction. The oil was dissolved in DCM (5 mL) under argon, and DIPEA (0.15 mL, 1.63 mmol, 12.5 eq) was added to the solution at 0 ˚C. The reaction mixture was allowed to stir for fifteen minutes. 3-cyano-4,5-difluorobenzenesulfonyl chloride **23** (40 mg, 0.17 mmol, 1.3 eq) in DCM (5 mL) was added to the reaction mixture. The ice bath was removed and the reaction was allowed to warm to room temperature. After two and a half hours saturated ammonium chloride solution was added. The mixture was extracted with dichloromethane (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting oil by silica gel chromatography (1:1 ethyl acetate/ hexanes) afforded (*R*)-1-((5-cyano-2,4-difluorophenyl)sulfonyl)-*N*-(4-cyanophenyl)-*N*-((5-cyclohexylpyridin-2-yl)methyl)azetidine-2-carboxamide **S3I-H275** (34 mg, 43% yield over two steps). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, J_{H-H} = 2.3 Hz 1H), 8.24 (t, J_{F-H} = 7.1 Hz, 1H), 7.71 (d, J_{H-H} = 8.2 Hz, 2H), 7.52 (dd, J_{H-H} = 8.1, 2.3 Hz, 1H), 7.38 (d, J_{H-H} = 8.2 Hz, 2H), 7.17 (d, J_{H-H} = 8.1 Hz, 1H), 7.12 (t, J_{H-H} = 8.8 Hz, 1H), 5.06 – 4.78 (m, 3H), 4.14 (q, J_{H-H} = 7.2 Hz, 2H), 4.02 – 3.90 (m, 1H), 2.59 – 2.46 (m, 1H), 2.42 – 2.27 (m, 1H), 2.03 – 1.94 (m, 1H), 1.93 – 1.72 (m, 5H), 1.49 – 1.30 (m, 5H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -92.87 (dd, J_{F-F} = 16.6 Hz, J_{F-H} = 8.1, 7.1 Hz, 1F), -94.3 (ddd, J_{F-F} = 16.6 Hz, J_{F-H} = 8.1, 7.1 Hz, 1 F); HPLC purity = 100%; HRMS (ESI) m/z = 598.1701 $[M + Na]^+$, HRMS (ESI+) calculated for $C_{30}H_{27}F_2N_5O_3S$: 575.1802, found 575.1808; EMSA IC_{50} 1.49 µM.



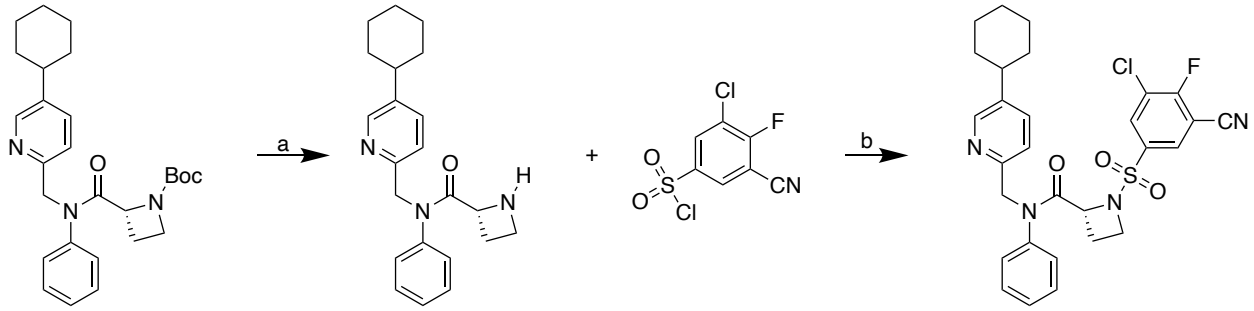
49 **50** **28** **S3I-H270**

Synthesis of S3I-H270. To a solution of *tert*-butyl (*R*)-2-(((5-cyclohexylpyridin-2-yl)methyl)(4-fluorophenyl)carbamoyl)azetidine-1-carboxylate **49** (220 mg, 0.47 mmol, 1.0 eq) in DCM (10 mL) was added TFA (2.0 mL, 26.0 mmol, 55.3 eq). The reaction mixture was stirred at room temperature for one hour. The reaction mixture was concentrated and the resulting oil was used directly in the next reaction. The oil was dissolved in DCM (5 mL), and DIPEA (0.5 mL, 2.99 mmol, 6.3 eq) was added to the solution at 0 ˚C. The reaction mixture was allowed to stir for fifteen minutes. 2-cyano-4,5-difluorobenzenesulfonyl chloride **28** (145 mg, 0.61 mmol, 1.3 eq) in DCM (5 mL) was added to the reaction mixture. The ice bath was removed and the reaction mixture was allowed to warm to room temperature. After two and a half hours saturated ammonium chloride solution was added. The mixture was extracted with dichloromethane (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting oil by silica gel chromatography (6:4 ethyl acetate/ hexanes) gave (*R*)-1-((2-cyano-4,5-difluorophenyl)sulfonyl)-*N*-((5-cyclohexylpyridin-2-yl)methyl)-*N*-(4-fluorophenyl)azetidine-2-carboxamide **S3I-H270** (100 mg, 38% yield over two steps). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, $J_{H\text{-}H}$ = 2.2 Hz, 1H), 7.97 (dd, $J_{F\text{-}H}$ = 9.4, 7.4 Hz, 1H), 7.67 (dd, $J_{F\text{-}H}$ = 9.1, 6.9 Hz, 1H), 7.53 (dd, $J_{H\text{-}H}$ = 8.1, 2.3 Hz, 1H), 7.22 (d, $J_{H\text{-}H}$ = 8.1 Hz, 1H), 7.17 (dd, $J_{F\text{-}H}$ = 4.9 Hz, $J_{H\text{-}H}$ = 9.0 Hz, 2H), 7.06 (dd, $J_{F\text{-}H}$ = 7.6 Hz, $J_{H\text{-}H}$ = 9.0 Hz, 2H), 5.08 – 4.82 (m, 3H), 4.25 – 4.15 (m, 1H), 3.99 (ddd, $J_{H\text{-}H}$ = 8.9, 7.2, 4.3 Hz, 1H), 2.59 – 2.47 (m, 1H), 2.40 – 2.26 (m, 1H), 1.91 – 1.74 (m, 6H), 1.49 – 1.29 (m, 5H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -111.46 (dd, $J_{F\text{-}H}$ = 8.0, 7.6 Hz, 1F), -124.57 (ddd, $J_{F\text{-}F}$ = 21.1 Hz, $J_{F\text{-}H}$ = 9.4, 6.9 Hz), -127.85 (ddd, $J_{F\text{-}F}$ = 21.1 Hz, $J_{F\text{-}H}$ = 9.1, 7.4 Hz); HPLC purity = 100% LCMS;

HRMS (ESI) m/z = 591.1660 $[M + Na]^+$, HRMS (ESI+) calculated for $C_{29}H_{27}F_3N_4O_3S$: 568.1756, found 568.1768; EMSA IC_{50} >20 μM.

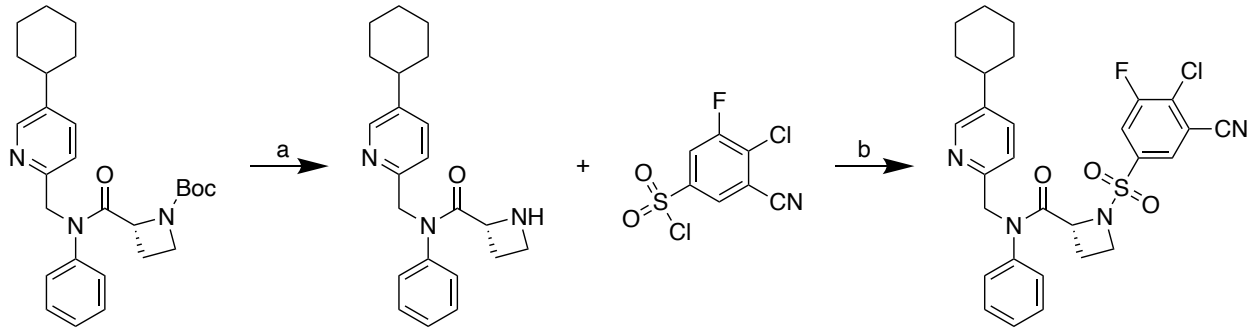


Synthesis of S3I-H265. To a solution of *tert*-butyl (*R*)-2-(((5-cyclohexylpyridin-2-yl)methyl) (phenyl)carbamoyl)azetidine-1-carboxylate **55** (50 mg, 0.11 mmol, 1.0 eq) in DCM (10 mL) was added TFA (0.5 mL, 6.53 mmol, 59.3 eq). The reaction mixture was stirred at room temperature for one hour. The reaction mixture was concentrated and the resulting oil was used directly in the next reaction. The oil was dissolved in DCM (5 mL), and DIPEA (0.1 mL, 0.57 mmol, 5.2 eq) was added to the solution at 0 ˚C. The reaction mixture was allowed to stir for fifteen minutes. 2-cyano-4,5-difluorobenzenesulfonyl chloride **28** (28 mg, 0.119 mmol, 1.1 eq) in DCM (5 mL) was added to the reaction mixture. The ice bath was removed and the reaction mixture was allowed to warm to room temperature. After two and a half hours saturated ammonium chloride solution was added. The mixture was extracted with dichloromethane (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting oil by silica gel chromatography (1:1 ethyl acetate/hexanes) afforded (*R*)-1-((2-cyano-4,5-difluorophenyl)sulfonyl)-*N*-((5-cyclohexylpyridin-2-yl)methyl)-*N*-phenylazetidine-2-carboxamide **S3I-H265** (22 mg, 36% yield over two steps) as a white solid. Spectroscopic Data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (d, J_{H-H} = 2.2 Hz, 1H), 7.96 (dd, J_{F-H} = 9.3, 7.2 Hz, 1H), 7.71 – 7.61 (m, 2H), 7.44 – 7.33 (m, 4H), 7.18 (d, J_{H-H} = 7.2 Hz, 2H), 5.19 – 4.93 (m, 3H), 4.17 (q, J_{H-H} = 7.9 Hz, 1H), 4.09 – 3.91 (m, 1H), 2.55 (m, 1H), 2.39 – 2.29 (m, 1H), 1.93 – 1.77 (m, 6H), 1.49 – 1.34 (m, 5H); ^{19}F NMR (471 MHz, $CDCl_3$) δ -124.50 (m, 1F), -127.80 (m, 1F); HPLC purity = 100%; HRMS (ESI) m/z = 573.1754 $[M + Na]^+$, HRMS (ESI+) calculated for $C_{29}H_{28}N_4O_3S$: 550.1850, found 550.1868; EMSA IC_{50} >30 μM.



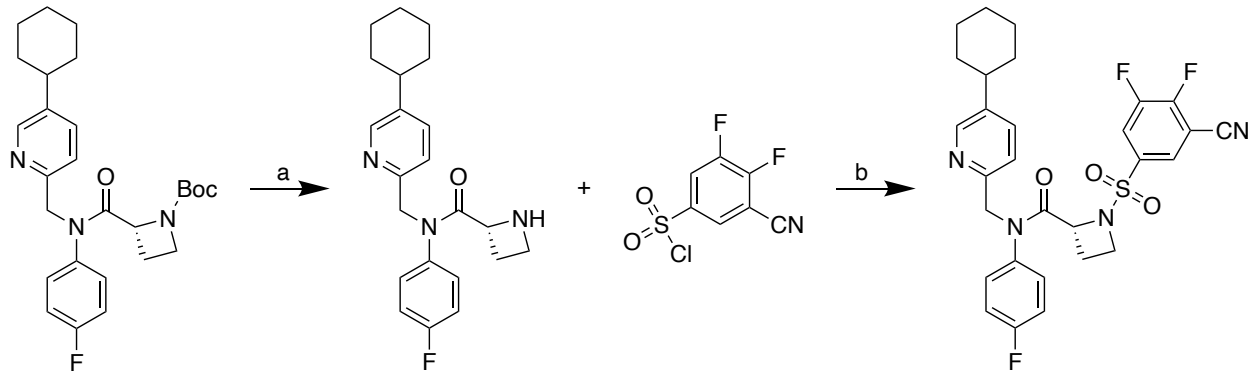
55 **56** **18** **S3I-H254**

Synthesis of S3I-H254. To a solution of *tert*-butyl (*R*)-2-(((5-cyclohexylpyridin-2-yl)methyl)(phenyl)carbamoyl)azetidine-1-carboxylate **55** (300 mg, 0.668 mmol, 1.0 eq) in DCM (10 mL) was added TFA (3.0 mL, 39.2 mmol, 61.6 eq). The reaction mixture was stirred at room temperature for one hour. The reaction mixture was concentrated and the resulting oil was used directly in the next reaction. The oil was dissolved in DCM (10 mL), and DIPEA (0.7 mL, 4.0 mmol, 6.0 eq) was added to the solution at 0 °C. The reaction mixture was allowed to stir for fifteen minutes. 3-chloro-5-cyano-4-fluorobenzenesulfonyl chloride **18** (200 mg, 0.78 mmol, 1.2 eq) in DCM (10 mL) was added. The ice bath was removed and the reaction was allowed to warm to room temperature. After two and a half hours saturated ammonium chloride solution was added. The reaction mixture was extracted with dichloromethane (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting oil by silica gel chromatography (1:1 ethyl acetate/hexanes) afforded (*R*)-1-((3-chloro-5-cyano-4-fluorophenyl)sulfonyl)-*N*-((5-cyclohexylpyridin-2-yl)methyl)-*N*-phenylazetidine-2-carboxamide **S3I-H254** (113 mg, 30% yield over two steps) as a white solid. Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (d, $J_{H\text{-}H}$ = 2.3 Hz, 1H), 8.33 (dd, $J_{F\text{-}H}$ = 6.5 Hz, $J_{H\text{-}H}$ = 2.2 Hz, 1H), 8.18 (dd, $J_{F\text{-}H}$ = 5.2 Hz, $J_{H\text{-}H}$ = 2.2 Hz, 1H), 7.56 (dd, $J_{H\text{-}H}$ = 8.1, 2.3 Hz, 1H), 7.45-7.35 (m, 3H), 7.32 (d, $J_{H\text{-}H}$ = 8.1 Hz, 1H), 7.23 – 7.11 (m, 2H), 5.12 – 4.89 (m, 3H), 3.96 (q, $J_{H\text{-}H}$ = 7.9 Hz, 1H), 3.69 (ddd, $J_{H\text{-}H}$ = 9.0, 7.0, 4.1 Hz, 1H), 2.53 (s, 1H), 2.45 – 2.29 (m, 1H), 1.93 – 1.76 (m, 6H), 1.51 – 1.24 (m, 5H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -101.02 (dd, $J_{F\text{-}H}$ = 6.5, 5.2 Hz, 1F); HPLC purity = 100%; HRMS (ESI) m/z = 589.1456 $[M + Na]^+$, HRMS (ESI+) calculated for $C_{29}H_{28}ClFN_4O_3S$: 566.1554, found 566.1566; EMSA IC_{50} 2.1 μM.



55 **56** **12** **S3I-H261**

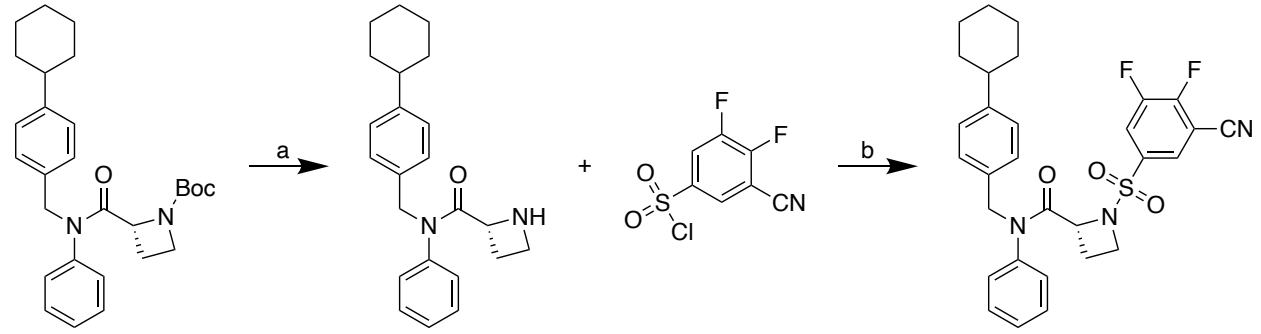
Synthesis of S3I-H261. To a solution of *tert*-butyl (*R*)-2-(((5-cyclohexylpyridin-2-yl)methyl)(phenyl)carbamoyl)azetidine-1-carboxylate **55** (50 mg, 0.1 mmol, 1.0 eq) in DCM (5 mL) was added TFA (0.5 mL, 6.5 mmol, 65.0 eq). The reaction mixture was stirred at room temperature for one hour. The reaction was concentrated and the resulting oil was used directly in the next reaction. The oil was dissolved in DCM (5 mL), and DIPEA (0.1 mL, 0.6 mmol, 6.0 eq) was added to the solution at 0 ˚C. The reaction mixture was allowed to stir for fifteen minutes. 4-chloro-3-cyano-5-fluorobenzenesulfonyl chloride **12** (30 mg, 0.12 mmol, 1.2 eq) in DCM (5 mL) was added. The ice bath was removed and the reaction mixture was allowed to warm to room temperature. After two and a half hours saturated ammonium chloride solution was added. The reaction mixture was extracted with dichloromethane (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting oil by silica gel chromatography (1:1 ethyl acetate/hexanes) afforded (*R*)-1-((4-chloro-3-cyano-5-fluorophenyl)sulfonyl)-*N*-((5-cyclohexylpyridin-2-yl)methyl)-*N*-phenylazetidine-2-carboxamide **S3I-H261** as a white solid (25 mg, 45% yield over two steps) as a white solid. Spectroscopic Data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.37 (d, $J_{H\text{-}H}$ = 2.3 Hz, 1H), 8.06 (m, 2H), 7.60 (d, $J_{H\text{-}H}$ = 8.3 Hz, 1H), 7.46 – 7.30 (m, 4H), 7.23 – 7.14 (m, 2H), 5.16 – 4.88 (m, 3H), 3.95 (q, $J_{H\text{-}H}$ = 8.0 Hz, 1H), 3.69 (td, $J_{H\text{-}H}$ = 8.0, 7.4, 4.0 Hz, 1H), 2.54 (m, 1H), 2.39 (m, 1H), 1.82 (m, 6H), 1.46 – 1.21 (m, 5H); ^{19}F NMR (471 MHz, $CDCl_3$) δ -106.79 (d, $J_{F\text{-}H}$ = 7.6 Hz, 1F); HPLC purity = 100%; EMSA IC_{50} > 100 μM.



49 **50** **6** **S3I-H241**

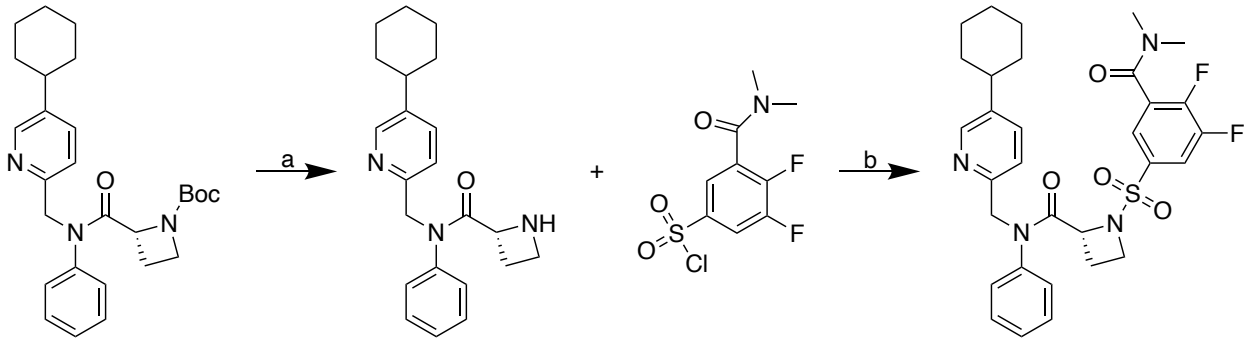
Synthesis of S3I-H241. To a solution of *tert*-butyl (*R*)-2-(((5-cyclohexylpyridin-2-yl)methyl)(4-fluorophenyl)carbamoyl)azetidine-1-carboxylate **49** (118 mg, 0.25 mmol, 1.0 eq) in DCM (10 mL) was added TFA (1.0 mL, 13.0 mmol, 52.0 eq). The reaction mixture was stirred at room temperature for one hour. The reaction mixture was concentrated and the resulting oil was used directly in the next reaction. The oil was dissolved in DCM (5 mL), and DIPEA (0.2 mL, 1.12 mmol, 4.5 eq) was added to the solution at 0 ˚C. The reaction mixture was allowed to stir for fifteen minutes. 3-cyano-4,5-difluorobenzenesulfonyl chloride **6** (77 mg, 0.32 mmol, 1.3 eq) in DCM (5.0 mL) was added. The ice bath was removed and the reaction mixture was allowed to warm to room temperature. After two and a half hours saturated ammonium chloride solution was added. The mixture was extracted with dichloromethane (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting oil by silica gel chromatography (1:1 ethyl acetate/hexanes) afforded(*R*)-1-((3-cyano-4,5-difluorophenyl)sulfonyl)-*N*-((5-cyclohexylpyridin-2-yl)methyl)-*N*-(4-fluorophenyl)azetidine-2-carboxamide **S3I-H241** (44 mg, 31% yield over two steps) as a white solid. Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (d, $J_{H\text{-}H}$ = 2.3 Hz, 1H), 8.21 (ddd, $J_{F\text{-}H}$ = 9.0, 7.0 Hz, $J_{H\text{-}H}$ = 2.2 Hz, 1H), 8.10 (ddd, $J_{F\text{-}H}$ = 4.9, 1.8 Hz, $J_{H\text{-}H}$ = 2.2 Hz 1H), 7.51 (dd, $J_{H\text{-}H}$ = 8.0, 2.3 Hz, 1H), 7.25 (d, $J_{H\text{-}H}$ = 8.0 Hz, 1H), 7.19 (dd, $J_{F\text{-}H}$ = 4.9 Hz, $J_{H\text{-}H}$ = 8.9 Hz, 2H), 7.08 (dd, $J_{F\text{-}H}$ = 8.1 Hz, $J_{H\text{-}H}$ = 8.9 Hz, 2H), 5.14 – 4.71 (m, 3H), 4.02 (dt, $J_{H\text{-}H}$ = 8.9, 7.5 Hz, 1H), 3.66 (ddd, $J_{H\text{-}H}$ = 8.9, 7.0, 4.1 Hz, 1H), 2.59 – 2.46 (m, 1H), 2.34 (ddt, $J_{H\text{-}H}$ = 10.8, 9.1, 7.9 Hz, 1H), 1.97 – 1.78 (m, 6H), 1.51 – 1.31 (m, 5H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -111.47 (tt, $J_{F\text{-}H}$ = 8.1, 4.8 Hz), -122.96 (ddd, $J_{F\text{-}F}$ = 19.9 Hz, $J_{F\text{-}H}$ = 7.0, 4.9 Hz), -129.97 (ddd, $J_{F\text{-}F}$ = 19.9 Hz, $J_{F\text{-}H}$

= 9.0, 1.8 Hz); HPLC purity = 95%; HRMS (ESI) m/z = 591.1640 $[M + Na]^+$, HRMS (ESI+) calculated for $C_{29}H_{27}F_3N_4O_3S$: 568.1756, found 568.1748; EMSA IC_{50} 2.2 ± 0.6 μM.



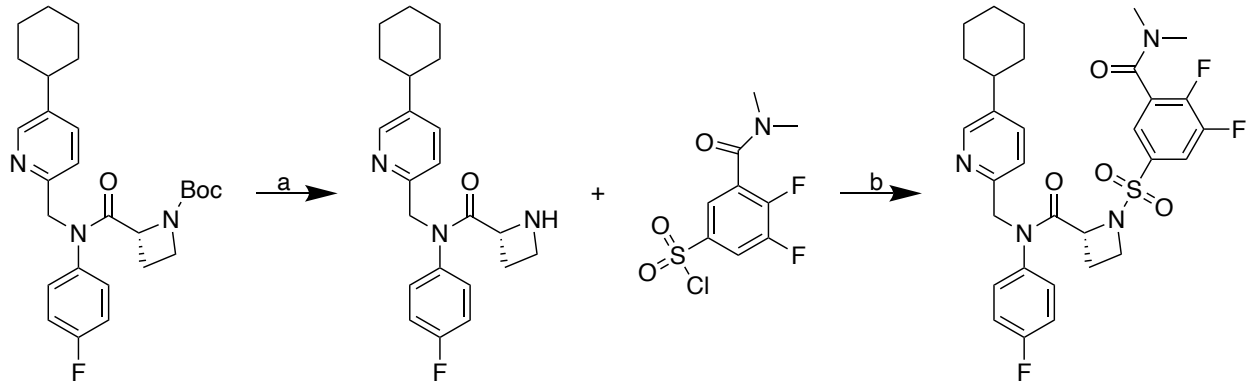
67 **68** **6** **S3I-H240**

Synthesis of S3I-H240. To a solution of *tert*-butyl (*R*)-2-((4-cyclohexylbenzyl) (phenyl)carbamoyl)azetidine-1-carboxylate **67** (108 mg, 0.24 mmol, 1.0 eq) in DCM (10 mL) was added TFA (1.0 mL, 13.0 mmol, 54.1 eq). The reaction mixture was stirred at room temperature for one hour. The reaction mixture was concentrated and the resulting oil was used directly in the next reaction. The oil was dissolved in DCM (5 mL), and DIPEA (0.25 mL, 1.45 mmol, 6.0 eq) was added to the solution at 0 ˚C. The reaction mixture was allowed to stir for fifteen minutes. 3-cyano-4,5-difluorobenzenesulfonyl chloride **6** (74 mg, 0.31 mmol, 1.3 eq) in DCM (5 mL) was added. The ice bath was removed and the reaction was allowed to warm to room temperature. After two and a half hours saturated ammonium chloride solution was added. The mixture was extracted with dichloromethane (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting oil by silica gel chromatography (1:1 ethyl acetate/hexanes) afforded 1-((3-cyano-4,5-difluorophenyl)sulfonyl)-*N*-(4-cyclohexylbenzyl)-*N*-phenylazetidine-2-carboxamide **S3I-H240** (52 mg, 41% yield over two steps) as a white solid. Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (ddd, $J_{F\text{-}H}$ = 9.1, 7.0 Hz, $J_{H\text{-}H}$ = 2.2 Hz, 1H), 8.06 (dt, $J_{F\text{-}H}$ = 5.0, 1.9 Hz, $J_{H\text{-}H}$ = 7.0 Hz 1H), 7.39 (m, 3H), 7.17 – 7.06 (m, 4H), 6.99 (dd, $J_{H\text{-}H}$ = 6.6, 2.9 Hz, 2H), 4.92 – 4.82 (m, 3H), 3.96 (dt, $J_{H\text{-}H}$ = 8.8, 7.5 Hz, 1H), 3.67 (ddd, $J_{H\text{-}H}$ = 9.1, 7.0, 4.1 Hz, 1H), 2.49 (dddd, $J_{H\text{-}H}$ = 14.4, 7.8, 6.1, 3.5 Hz, 1H), 2.30 (ddt, $J_{H\text{-}H}$ = 10.9, 9.1, 7.9 Hz, 1H), 1.93 – 1.69 (m, 5H), 1.49 – 1.20 (m, 5H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -123.00 (ddd, $J_{F\text{-}F}$ = 19.9 Hz, $J_{F\text{-}H}$ = 7.0 , 5.0 Hz, 1F), -129.92 (ddd, $J_{F\text{-}F}$ = 19.9 Hz, $J_{F\text{-}H}$ = 9.1, 1.9 Hz, 1F); HPLC purity = 100%; HRMS (ESI) m/z = 572.1787 $[M + Na]^+$, HRMS (ESI+) calculated for $C_{29}H_{29}F_2N_3O_3S$: 549.1897, found 549.1893; EMSA IC_{50} 3.3 ± 0.2 μM.



Synthesis of S3I-H296. To a solution of *tert*-butyl (*R*)-2-(((5-cyclohexylpyridin-2-yl)methyl)(phenyl)carbamoyl)azetidine-1-carboxylate **55** (133 mg, 0.29 mmol, 1.0 eq) in DCM (10 mL) was added TFA (1.0 mL, 13.0 mmol, 45.0 eq). The reaction mixture was stirred at room temperature for one hour. The reaction was then concentrated and the resulting oil was used directly in the next reaction. The oil was dissolved in DCM (10 mL), and DIPEA (0.3 mL, 1.8 mmol, 6.0 eq) was added to the solution at 0 °C. The reaction mixture was allowed to stir for fifteen minutes and then 3-(dimethylcarbamoyl)-4,5-difluorobenzenesulfonyl chloride **31** (169 mg, 0.7 mmol, 2.3 eq) in DCM (10 mL) was added. The ice bath was removed and the reaction mixture was allowed to warm to room temperature. After two and a half hours saturated ammonium chloride solution was added. The mixture was extracted with dichloromethane (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting oil by silica gel chromatography (27:63 acetone/hexanes) gave (*R*)-*N*-((5-cyclohexylpyridin-2-yl)methyl)-1-((3-(dimethylcarbamoyl)-4,5-difluorophenyl)sulfonyl)-*N*-phenylazetidine-2-carboxamide **S3I-H296** as a white solid (3 mg, 2% yield over two steps at 100% purity). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, $J_{H\text{-}H}$ = 2.3 Hz, 1H), 7.89 (ddd, $J_{F\text{-}H}$ = 9.2, 7.0 Hz, $J_{H\text{-}H}$ = 2.3 Hz, 1H), 7.72 (dt, $J_{F\text{-}H}$ = 4.3, 1.9 Hz, 1H), 7.53 (dd, $J_{H\text{-}H}$ = 8.0, 2.3 Hz, 1H), 7.30 (d, $J_{H\text{-}H}$ = 8.0 Hz, 1H), 7.20 – 7.03 (m, 4H), 4.97 (s, 2H), 4.83 (t, $J_{H\text{-}H}$ = 8.3 Hz, 1H), 3.87 (q, $J_{H\text{-}H}$ = 8.1 Hz, 1H), 3.69 (ddd, $J_{H\text{-}H}$ = 8.9, 7.2, 4.0 Hz, 1H), 3.14 (s, 3H), 2.94 (s, 3H), 2.52 (m, 1H), 2.38 (dq, $J_{H\text{-}H}$ = 10.4, 8.2 Hz, 1H), 2.15 (m, 1H), 1.92 – 1.73 (m, 5H), 1.47 – 1.30 (m, 5H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -131.69 (ddd, $J_{F\text{-}F}$ = 22.1 Hz, $J_{F\text{-}H}$ = 7.0, 4.3 Hz, 1F), -132.10 (ddd, $J_{F\text{-}F}$ = 22.1 Hz, $J_{F\text{-}H}$ = 9.2, 1.9 Hz, 1F); HPLC

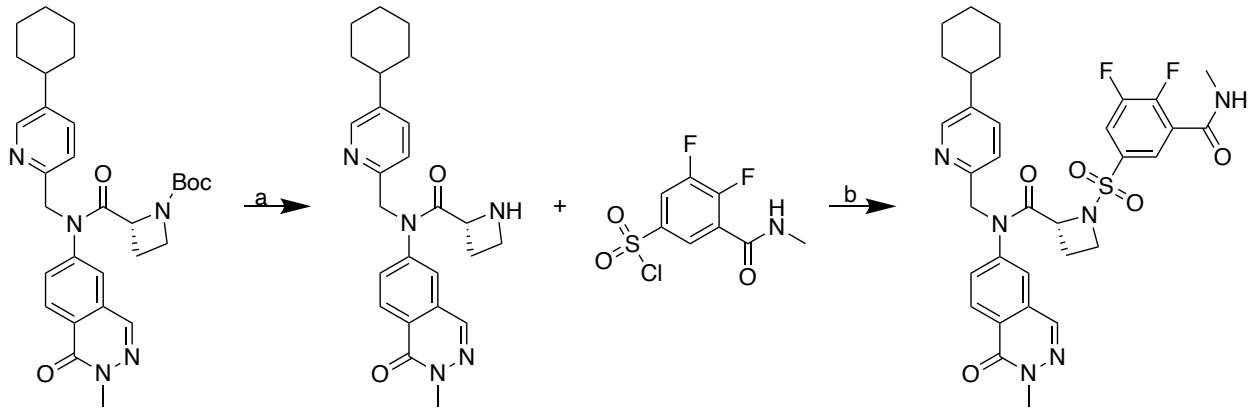
purity = 100%; HRMS (ESI) m/z = 619.2176 $[M + Na]^+$, HRMS (ESI+) calculated for $C_{31}H_{34}F_2N_4O_4S$: 596.2268, found 596.2285; EMSA IC_{50} >20 μM.



49 **50** **31** **S3I-H299**

Synthesis of S3I-H299. To a solution of *tert*-butyl (*R*)-2-(((5-cyclohexylpyridin-2-yl)methyl)(4-fluorophenyl)carbamoyl)azetidine-1-carboxylate **49** (200 mg, 0.42 mmol, 1.0 eq) in DCM (10 mL) was added TFA (2.0 mL, 26.0 mmol, 62.0 eq). The reaction mixture was stirred at room temperature for one hour. The reaction was concentrated and the resulting solid was used directly in the next reaction. The solid was dissolved in DCM (10 mL), and DIPEA (0.5 mL, 2.97 mmol, 7.0 eq) was added to the solution at 0 ˚C. The reaction mixture was allowed to stir for fifteen minutes. 3-(dimethylcarbamoyl)-4,5-difluorobenzenesulfonyl chloride **31** (157 mg, 0.55 mmol, 1.3 eq) in DCM (10 mL) was added. The ice bath was removed and the reaction mixture was allowed to warm to room temperature. After two and a half hours saturated ammonium chloride solution was added. The mixture was extracted with dichloromethane (x3). The combined organic extracts were washed with water, brine, and dried over Na_2SO_4. Purification of the resulting oil by silica gel chromatography (4:1 ethyl acetate/hexanes) afforded (*R*)-*N*-((5-cyclohexylpyridin-2-yl)methyl)-1-((3-(dimethylcarbamoyl)-4,5-difluorophenyl)sulfonyl)-*N*-(4-fluorophenyl)azetidine-2-carboxamide **S3I-H299** as a white solid (125 mg, 48 % yield over two steps). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, $J_{H\text{-}H}$ = 2.3 Hz, 1H), 7.89 (ddd, $J_{F\text{-}H}$ = 9.2 Hz, 7.0, $J_{H\text{-}H}$ = 2.3 Hz, 1H), 7.72 (dt, $J_{F\text{-}H}$ = 4.3, 1.9 Hz, 1H), 7.53 (dd, $J_{H\text{-}H}$ = 8.1, 2.3 Hz, 1H), 7.30 (d, $J_{H\text{-}H}$ = 8.1 Hz, 1H), 7.16 (dd, $J_{F\text{-}H}$ = 4.8 Hz, $J_{H\text{-}H}$ = 8.5 Hz, 2H), 7.07 (dd, $J_{F\text{-}H}$ = 8.1 Hz, $J_{H\text{-}H}$ = 8.5 Hz, 2H), 4.97 (s, 2H), 4.83 (t, $J_{H\text{-}H}$ = 8.3 Hz, 1H), 3.87 (q, $J_{H\text{-}H}$ = 8.2 Hz, 1H), 3.69 (ddd, $J_{H\text{-}H}$ = 8.9, 7.2, 4.0 Hz, 1H), 3.14 (s, 3H), 2.94 (s, 3H), 2.59 – 2.46 (m, 1H), 2.38 (dq, $J_{H\text{-}H}$ = 10.4, 8.2 Hz, 1H), 2.19 – 2.09 (m, 1H), 1.92 – 1.73 (m, 6H), 1.47 – 1.30 (m, 5H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -111.56 (dd, $J_{F\text{-}H}$ = 8.1, 4.8 Hz, 1F), -131.59 (ddd, $J_{F\text{-}F}$ = 21.4 Hz, $J_{F\text{-}H}$ = 9.2, 4.3 Hz, 1F), -132.16 (ddd, $J_{F\text{-}F}$ = 21.4 Hz, $J_{F\text{-}H}$ = 7.0, 1.9 Hz, 1F); HRMS (ESI) m/z =

637.2083 $[M + Na]^+$, HRMS (ESI+) calculated for $C_{31}H_{33}F_3N_4O_4S$: 614.2174, found 614.2191; EMSA IC_{50} >20 μM.



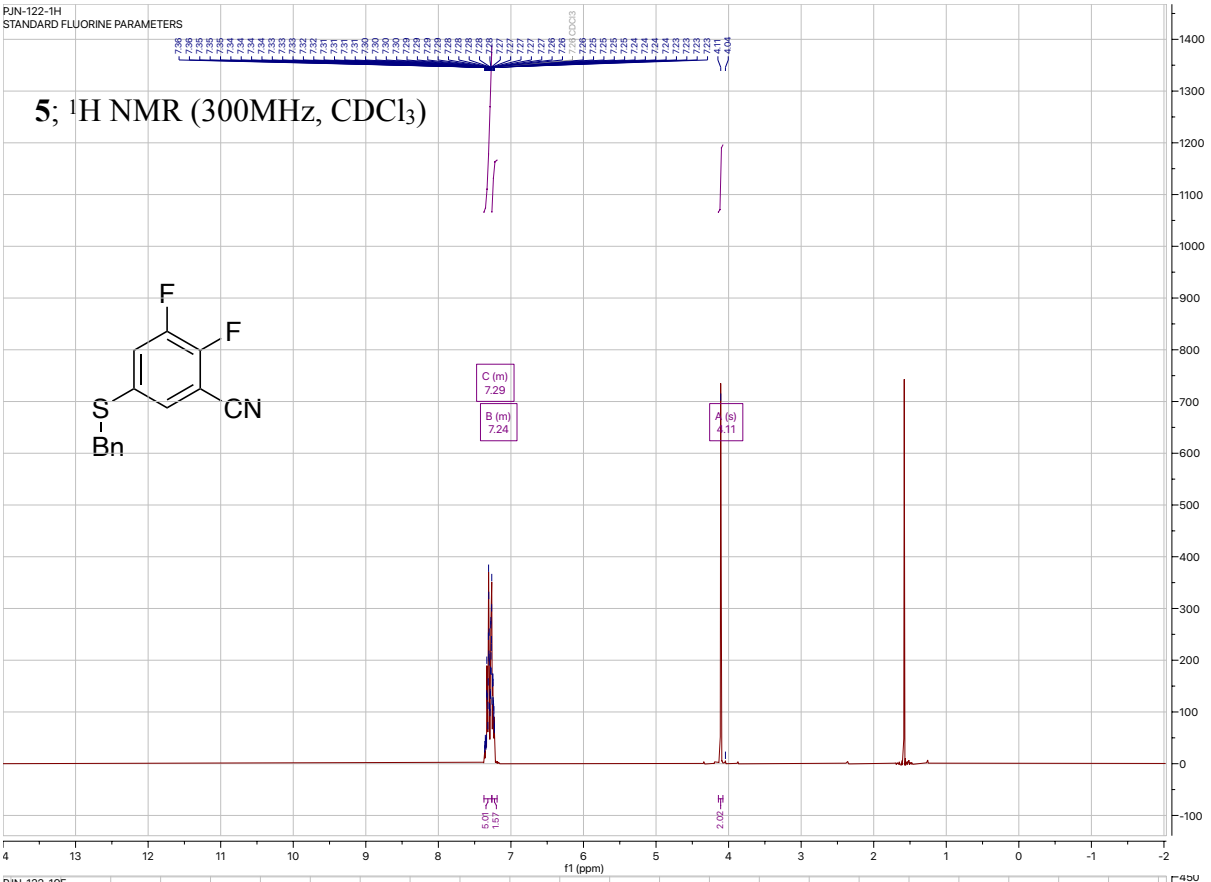
73 **74** **34** **S3I-H308**

Synthesis of S3I-H308. To **73** (120 mg, 0.22 mmol, 1.0 eq), in DCM (10 mL), was added TFA (1.0 mL, 13.0 mmol, 59.0 eq). The reaction was allowed to stir for one hour. The reaction solvent was removed *in vacuo*. The resulting oil was dissolved in DCM (5 mL), and cooled in an ice bath. After five minutes DIPEA (0.2 mL, 1.3 mmol, 6.1 eq) was added to the solution, and it was allowed to stir for fifteen minutes. 3,4-difluoro-5-(methylcarbamoyl)benzenesulfonyl chloride **34** (85 mg, 0.31 mmol, 1.4 eq), was added as a solution in DCM (5 mL). The ice bath was removed and the reaction mixture was allowed to reach room temperature. After one and a half hours at room temperature, saturated ammonium chloride solution was added. The resulting aqueous phase was extracted with DCM (x3). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. Purification of the resulting oil by silica gel chromatography (85:15 ethyl acetate/hexanes) gave the desired product (*R*)-*N*-((5-cyclohexylpyridin-2-yl)methyl)-1-((3,4-difluoro-5-(methylcarbamoyl)phenyl)sulfonyl)-*N*-(2-methyl-1-oxo-1,2-dihydrophthalazin-6-yl)azetidine-2-carboxamide **S3I-H308** as a white solid (36 mg, 25% yield over two steps). Spectroscopic Data: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (d, $J_{H\text{-}H}$ = 8.4 Hz, 1H), 8.38 – 8.21 (m, 2H), 8.13 (s, 1H), 7.92 (ddd, $J_{F\text{-}H}$ = 9.1, 7.1 Hz, $J_{H\text{-}H}$ = 2.2 Hz, 1H), 7.65 (d, $J_{H\text{-}H}$ = 2.0 Hz, 1H), 7.55 (ddd, $J_{F\text{-}H}$ = 7.6, 1.8 Hz, $J_{H\text{-}H}$ = 2.2 Hz, 2H), 6.66 (d, $J_{H\text{-}H}$ = 4.3 Hz, 1H), 5.10 – 4.82 (m, 3H), 3.87 (m, 4H), 3.71 (s, 1H), 3.04 (dd, $J_{H\text{-}H}$ = 4.9, 0.8 Hz, 3H), 2.58 – 2.32 (m, 2H), 1.91 – 1.75 (m, 6H), 1.50 – 1.31 (m, 5H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -131.91 (ddd, $J_{F\text{-}F}$ = 21.7 Hz, $J_{F\text{-}H}$ = 7.6, 7.1 Hz, 1F), -132.64 (ddd, $J_{F\text{-}F}$ = 21.7 Hz, $J_{F\text{-}H}$ = 9.1, 1.8 Hz, 1F); HPLC purity = 96%; HRMS (ESI) m/z = $[M + Na]^+$, HRMS (ESI+) calculated for $C_{33}H_{34}F_2N_6O_5S$: 664.2279, found 664.2263; EMSA IC_{50} 13.3 μM.

Appendix I

Selected Spectra for Compounds from Chapter 2

Table of Contents for Appendix I


PJN-122-1H
STANDARD FLUORINE PARAMETERS
5; 1H NMR (300MHz, CDCl3)
F
F
S
CN
Bn
C (m)
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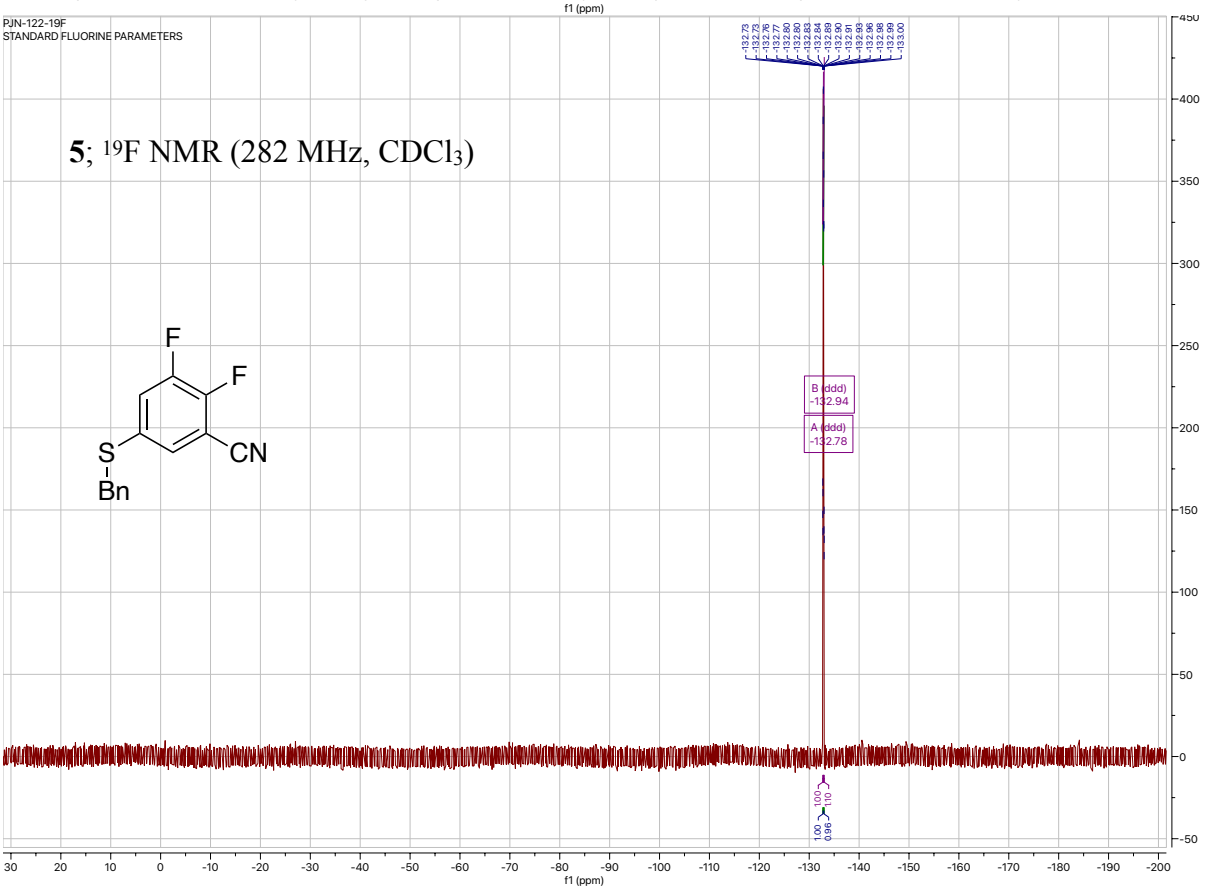
B (m)
7.24
A (s)
4.11
f1 (ppm)


PJN-122-19F
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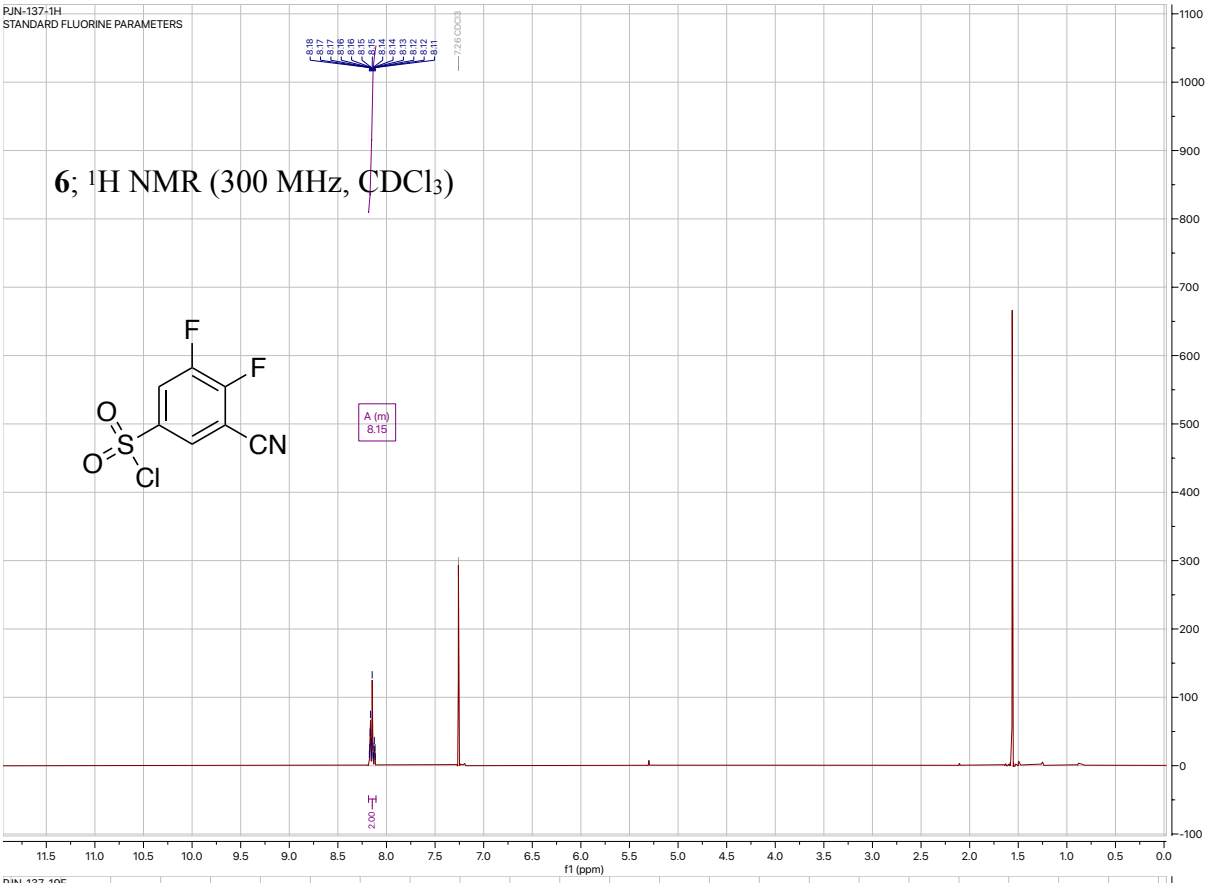
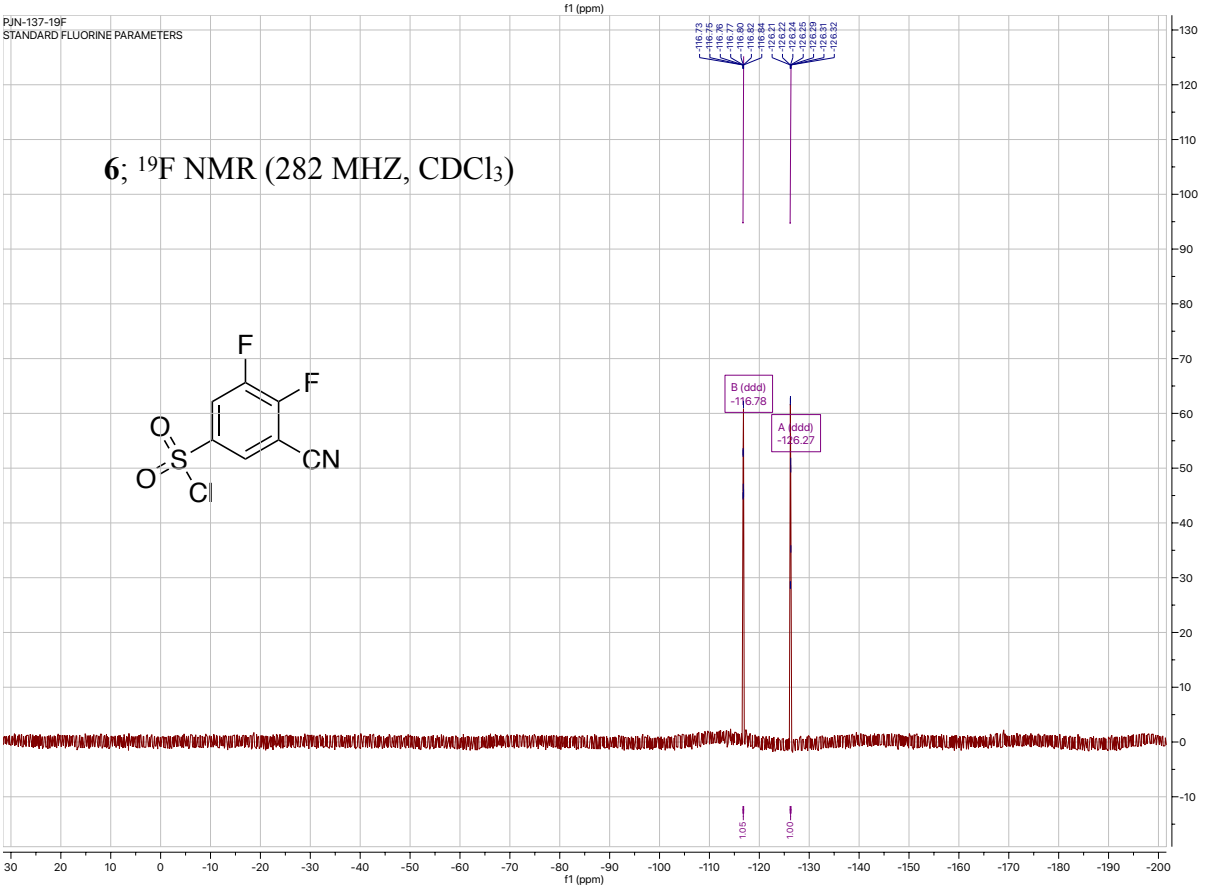
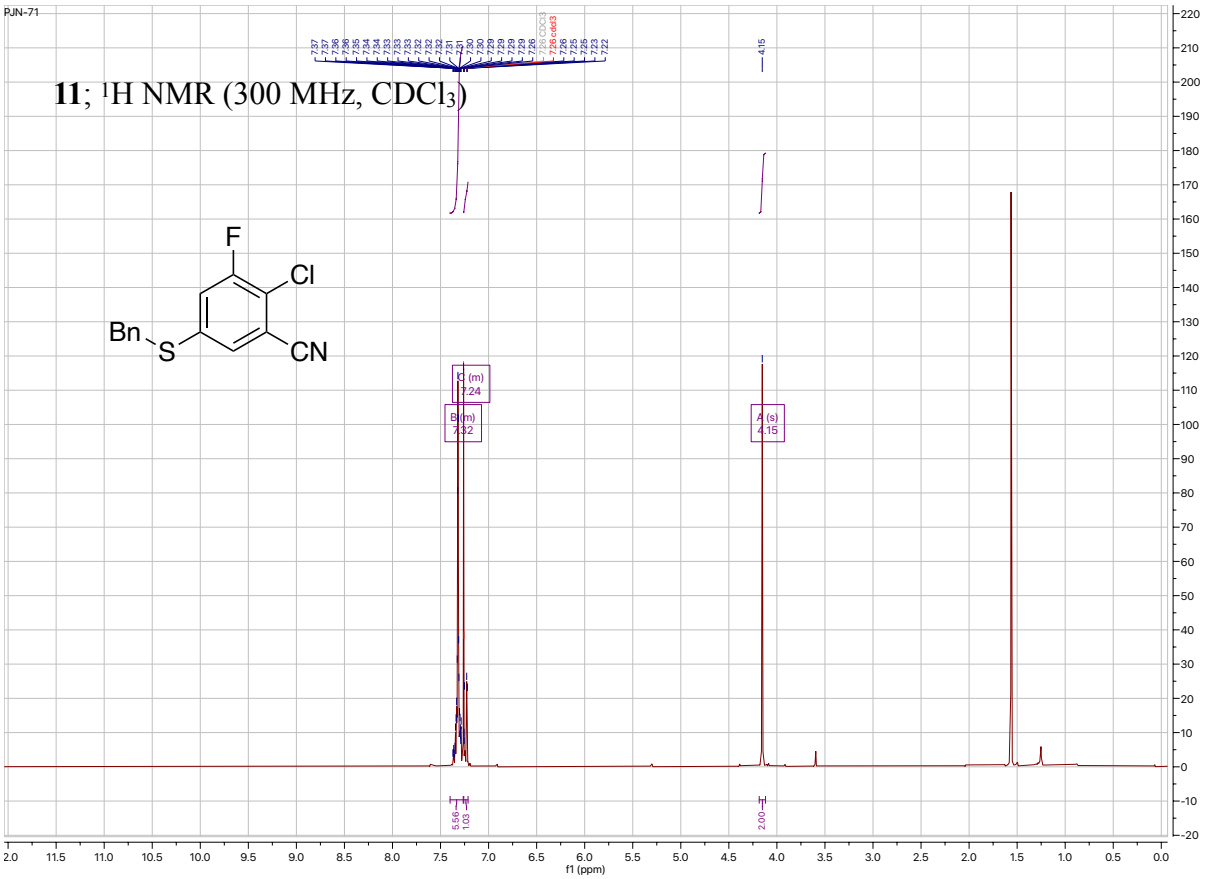
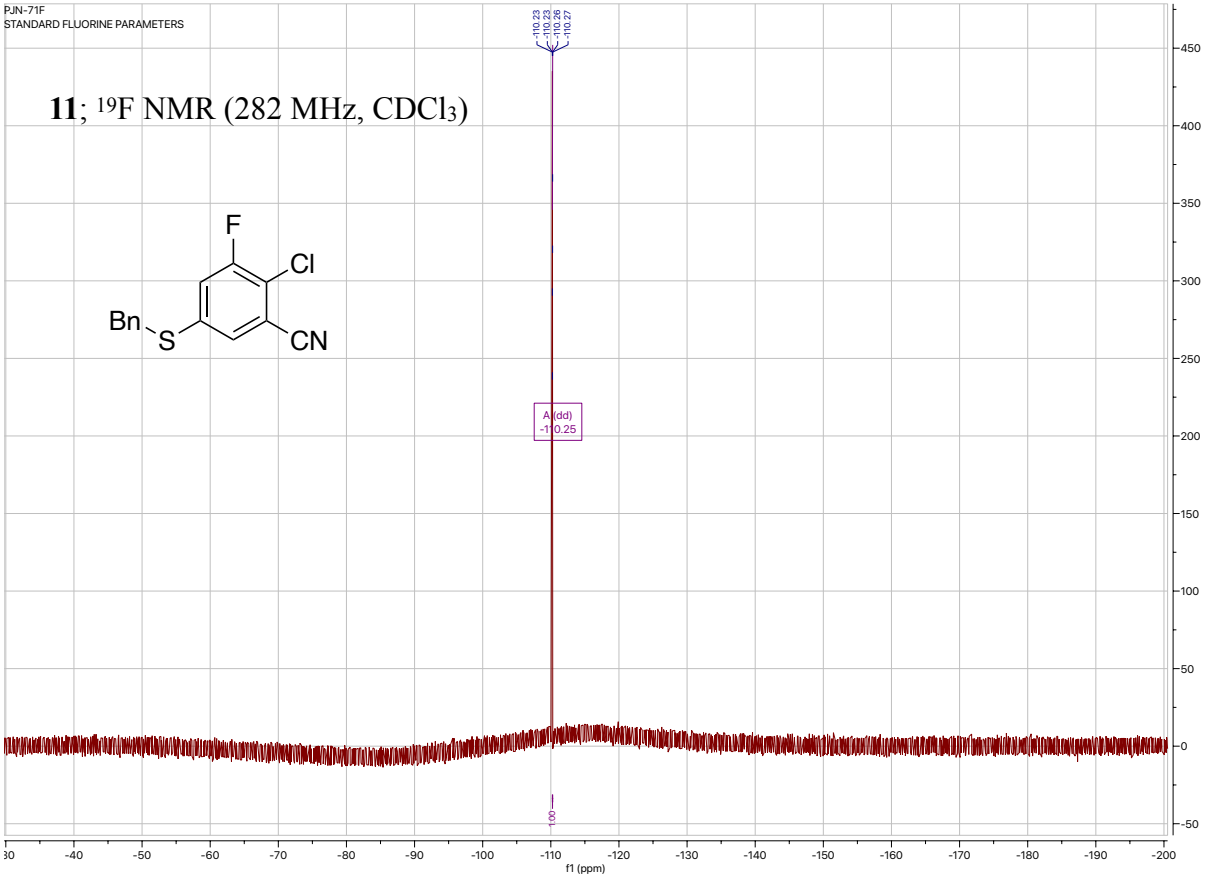
5; 19F NMR (282 MHz, CDCl3)
F
F
S
CN
Bn
B (ddd)
-132.94
A (ddd)
-132.78
f1 (ppm)


PJN-137-1H
STANDARD FLUORINE PARAMETERS
6; 1H NMR (300 MHz, CDCl3)
F
F
O
S
O
Cl
CN
A (m)
8.15
2.00
f1 (ppm)


PJN-137-19F
STANDARD FLUORINE PARAMETERS
6; 19F NMR (282 MHZ, CDCl3)
F
F
O
S
O
Cl
CN
B (ddd)
-116.78
A (ddd)
-126.27
1.05
1.00
f1 (ppm)


PJN-71
11; 1H NMR (300 MHz, CDCl3)
F
Cl
Bn
S
CN
f1 (ppm)


PJN-71F
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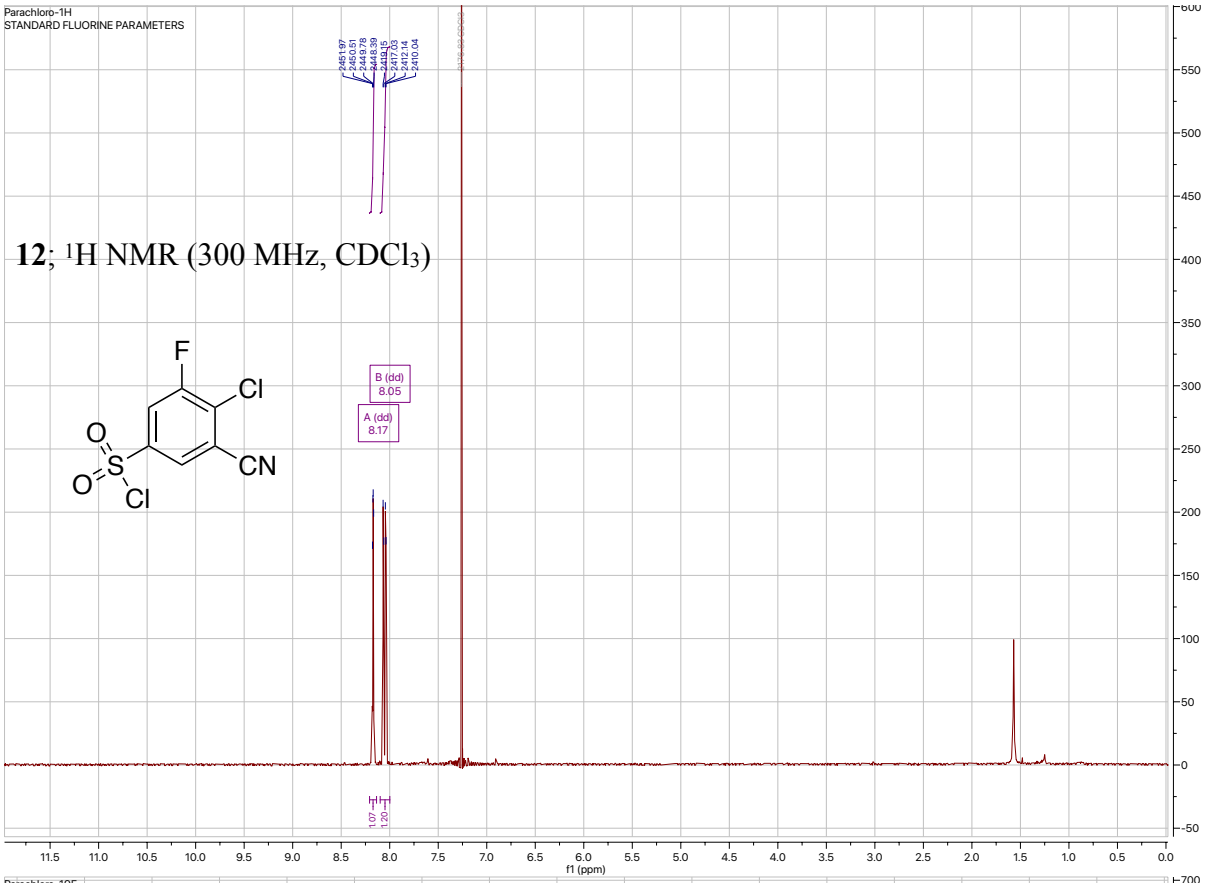
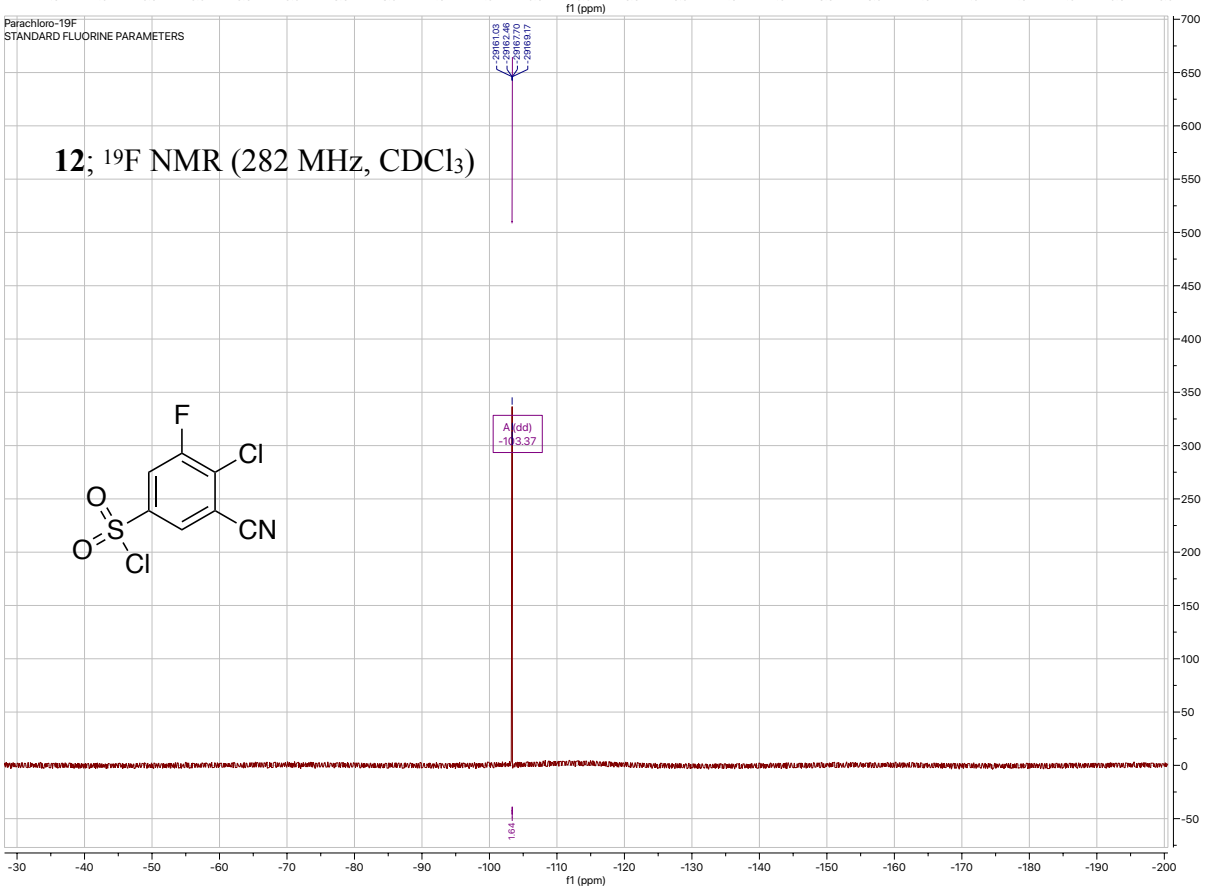
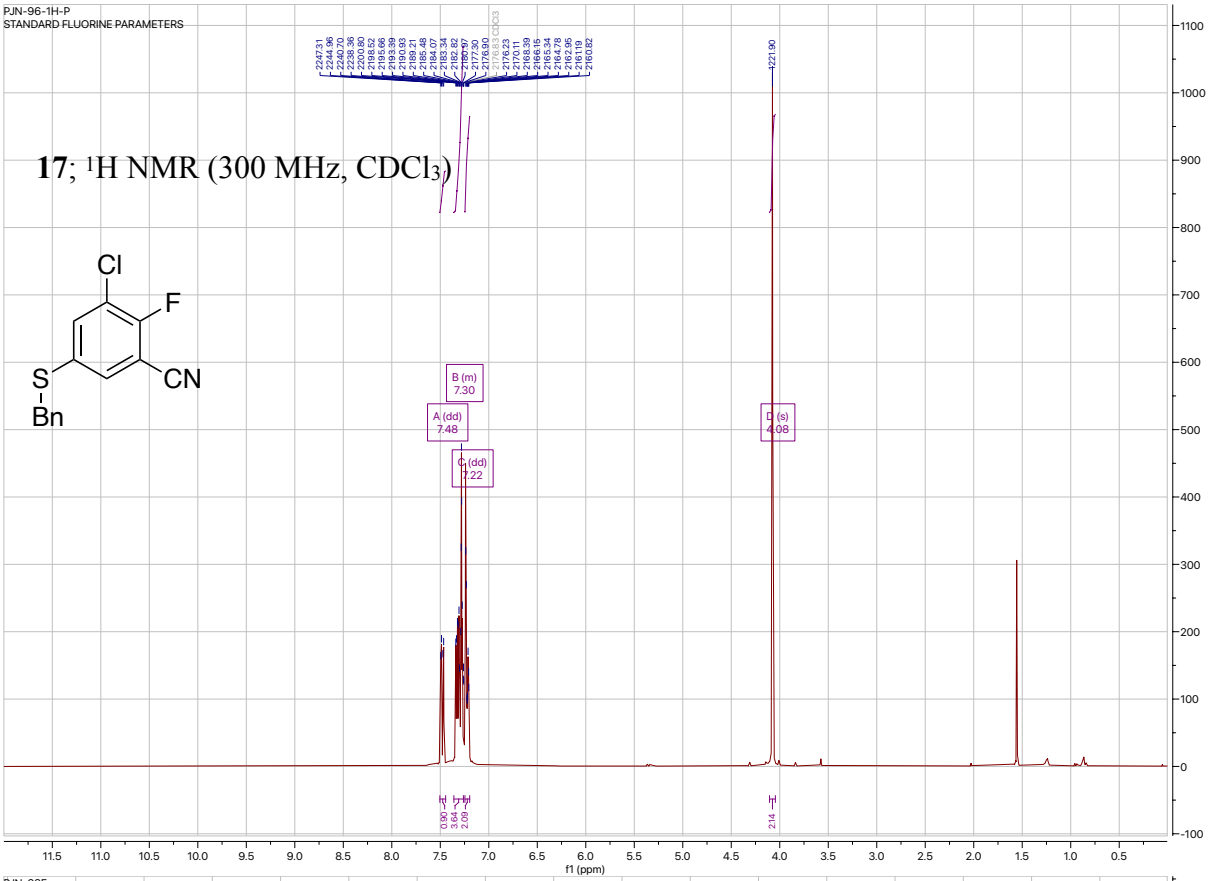
11; 19F NMR (282 MHz, CDCl3)
F
Cl
Bn
S
CN
f1 (ppm)



12; ^{1}H NMR (300 MHz, $CDCl_3$)



12; ^{19}F NMR (282 MHz, $CDCl_3$)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STANDARD FLUORINE PARAMETERS
17; 1H NMR (300 MHz, CDCl3)
Cl
F
S
CN
Bn
B (m)
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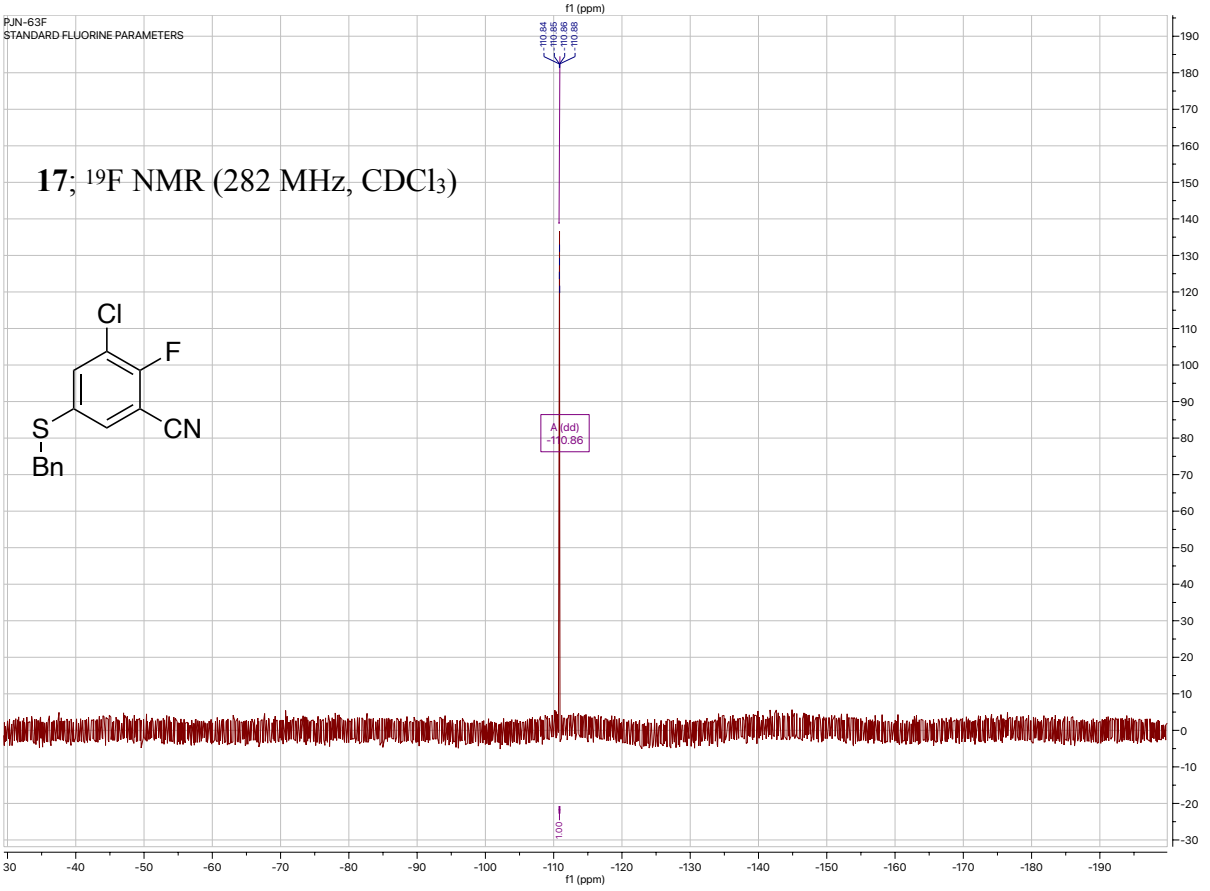
A (dd)
7.48
C (dd)
7.22
D (s)
4.08
f1 (ppm)


PJN-63F
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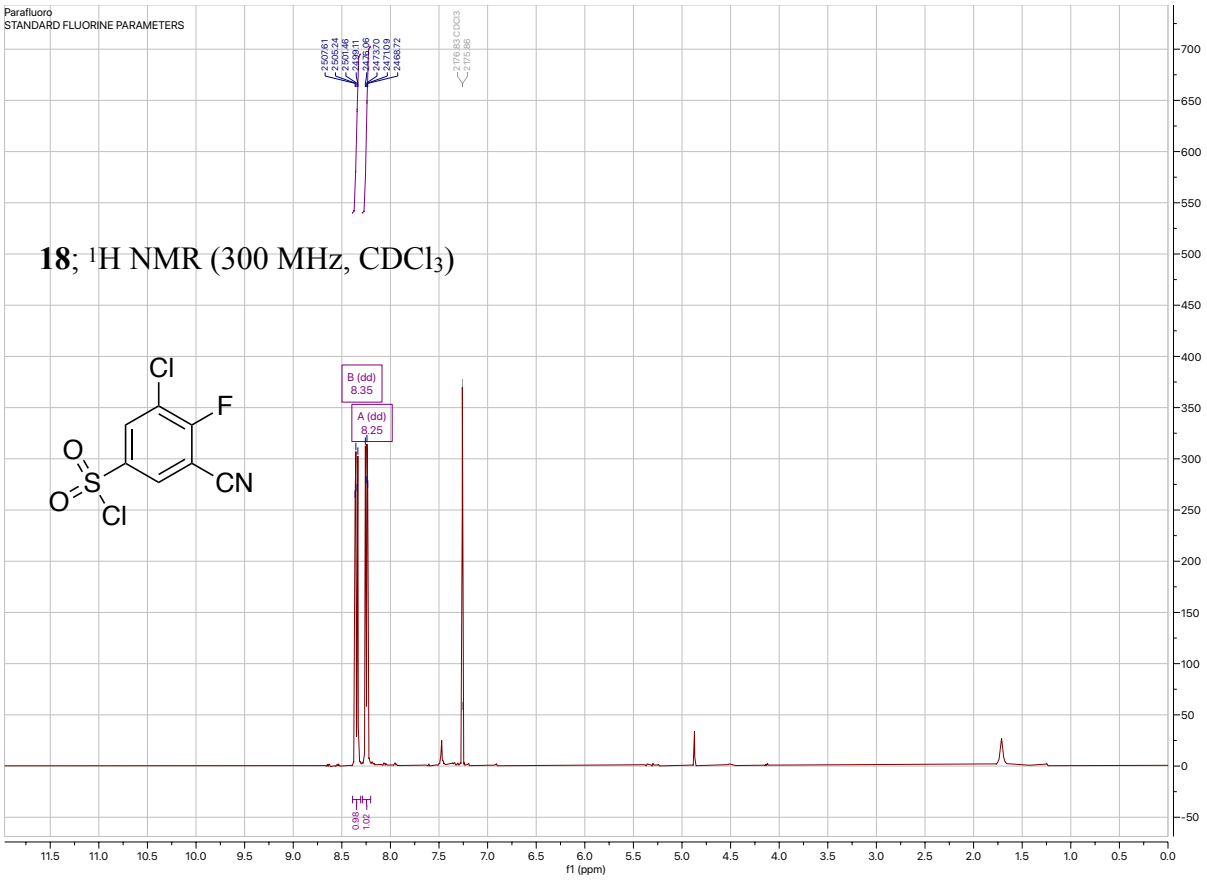
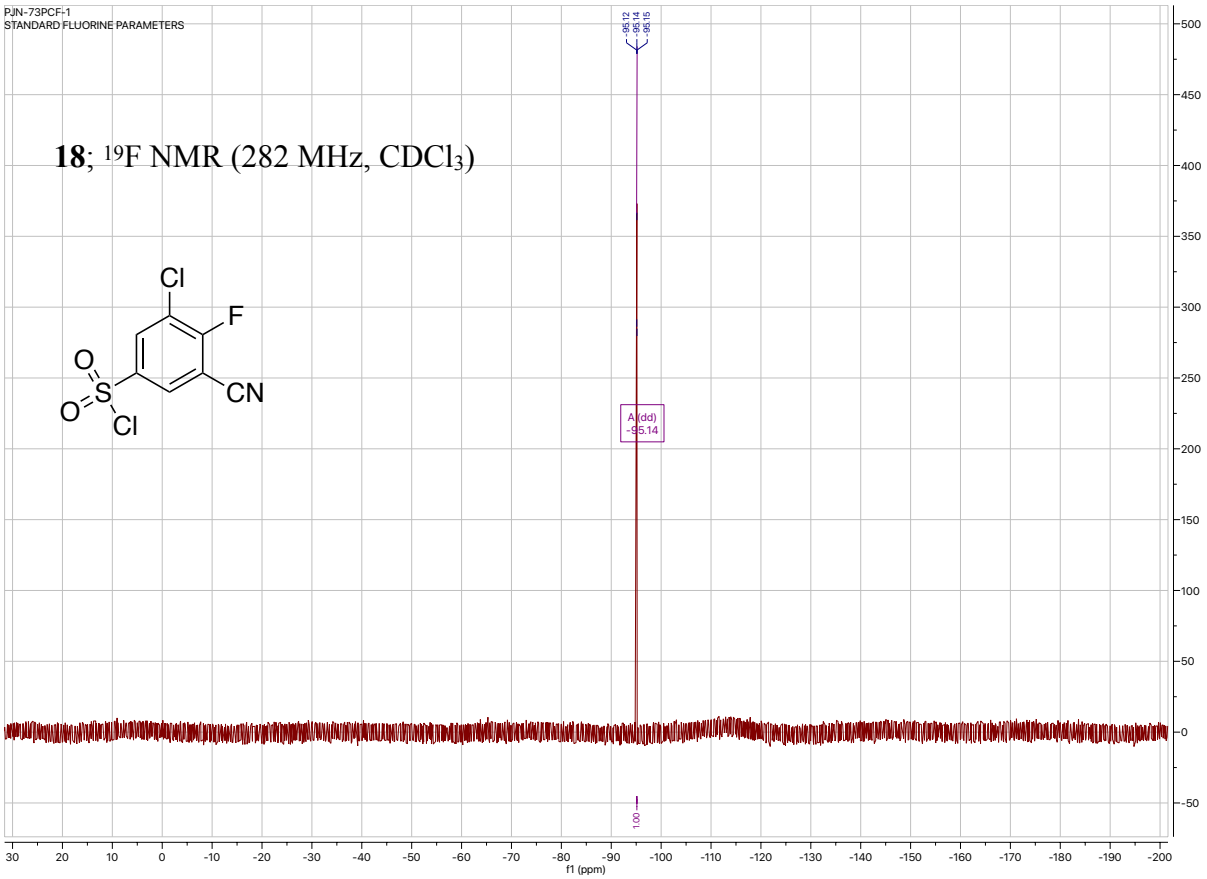
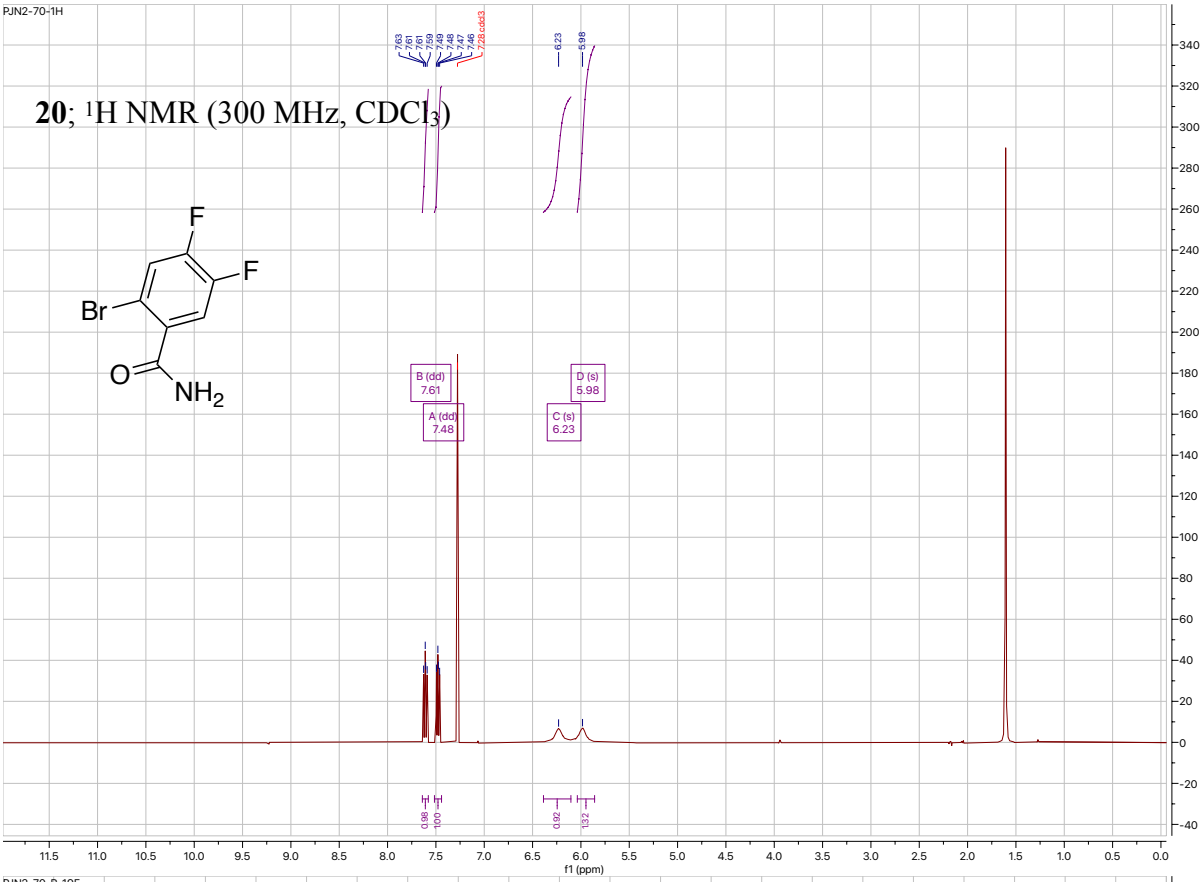
F
S
CN
Bn
A (dd)
-110.86
f1 (ppm)

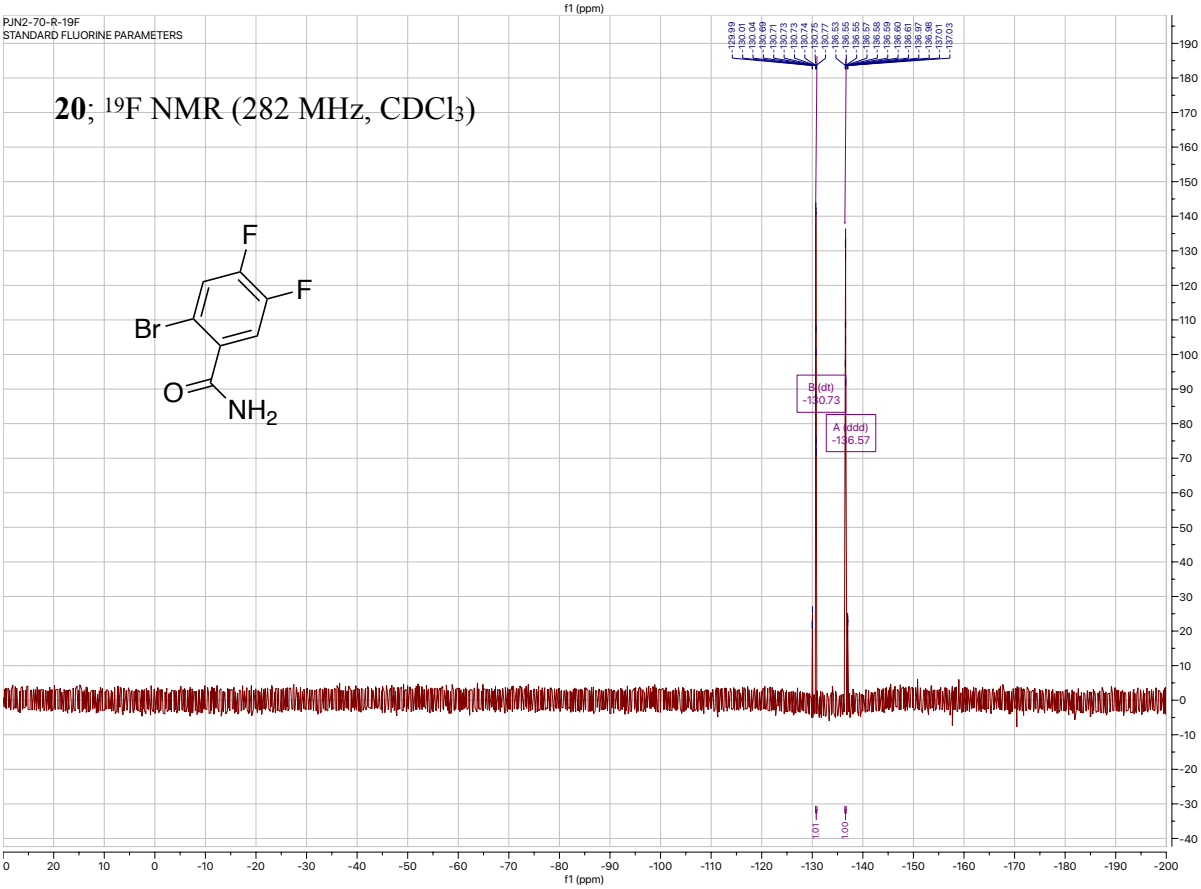


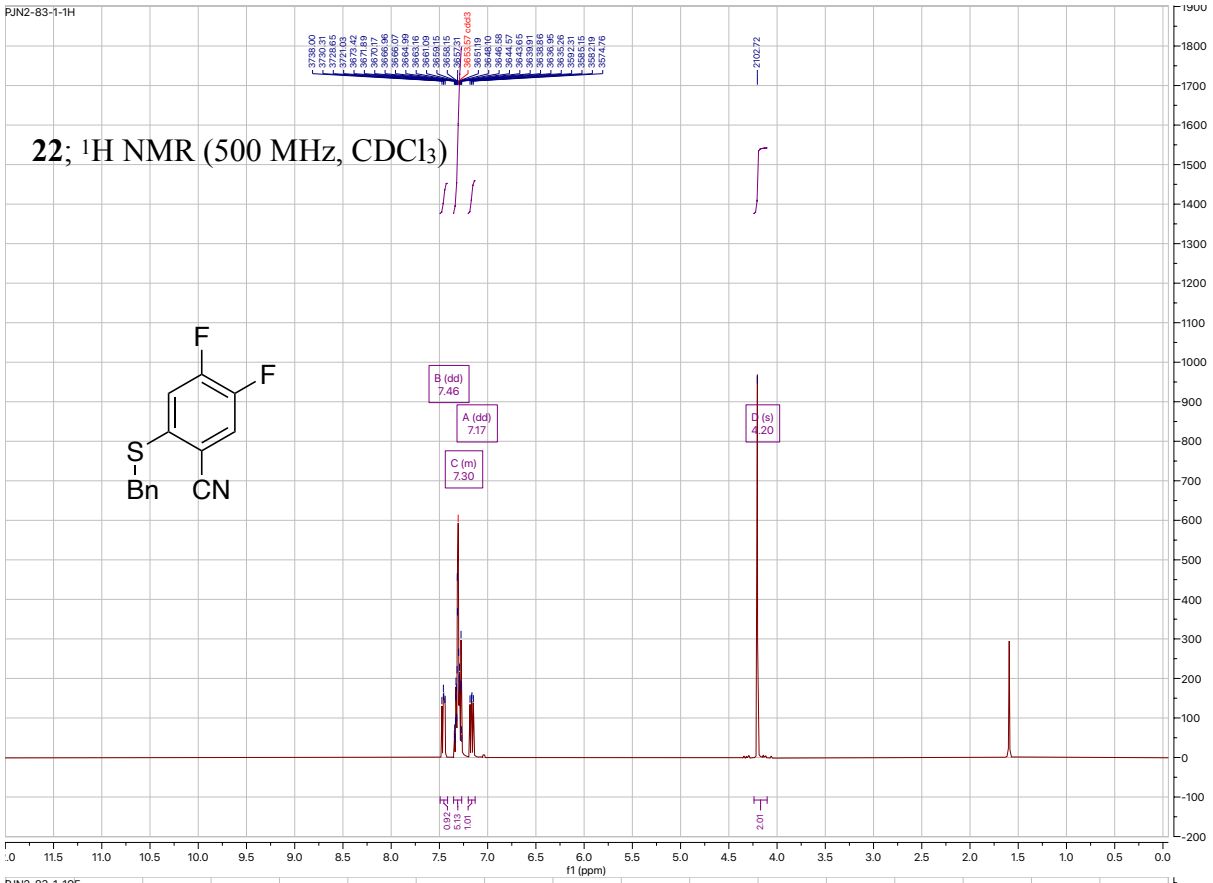
18; ^{1}H NMR (300 MHz, $CDCl_3$)

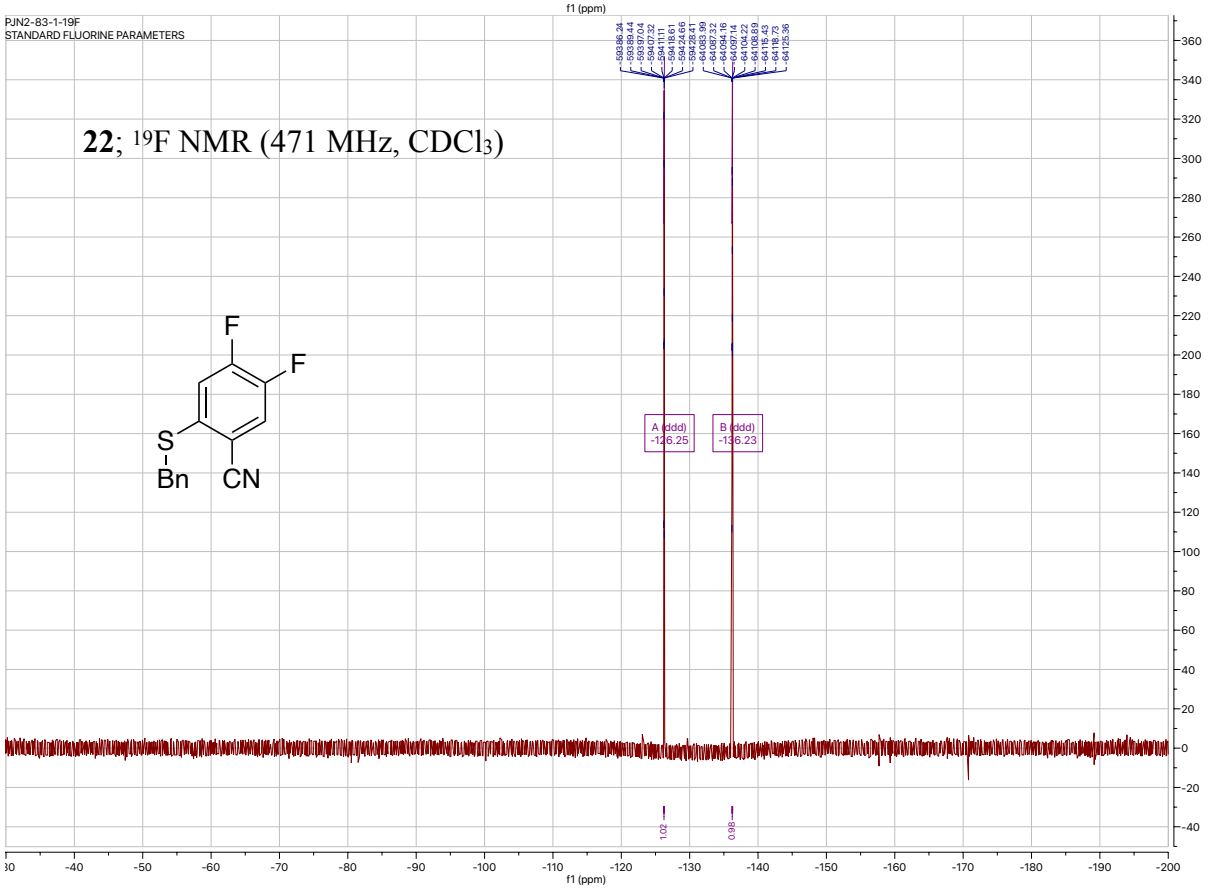


18; ^{19}F NMR (282 MHz, $CDCl_3$)

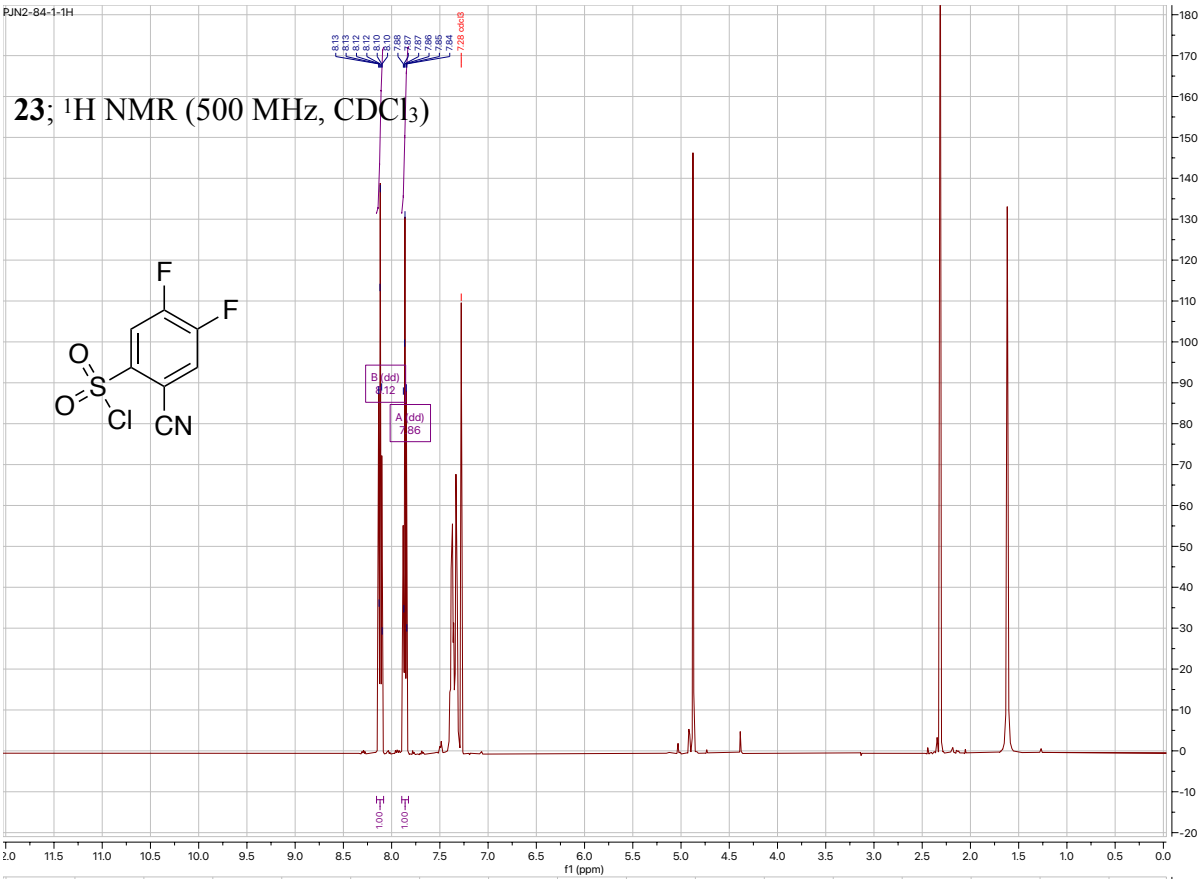

PJN2-70-1H
20; 1H NMR (300 MHz, CDCl3)
F
F
Br
O
NH2
B (dd)
7.61
A (dd)
7.48
D (s)
5.98
C (s)
6.23
f1 (ppm)


PJN2-70-R-19F
STANDARD FLUORINE PARAMETERS
20; 19F NMR (282 MHz, CDCl3)
F
F
Br
O
NH2
B (dt)
-130.73
A (ddd)
-136.57
f1 (ppm)

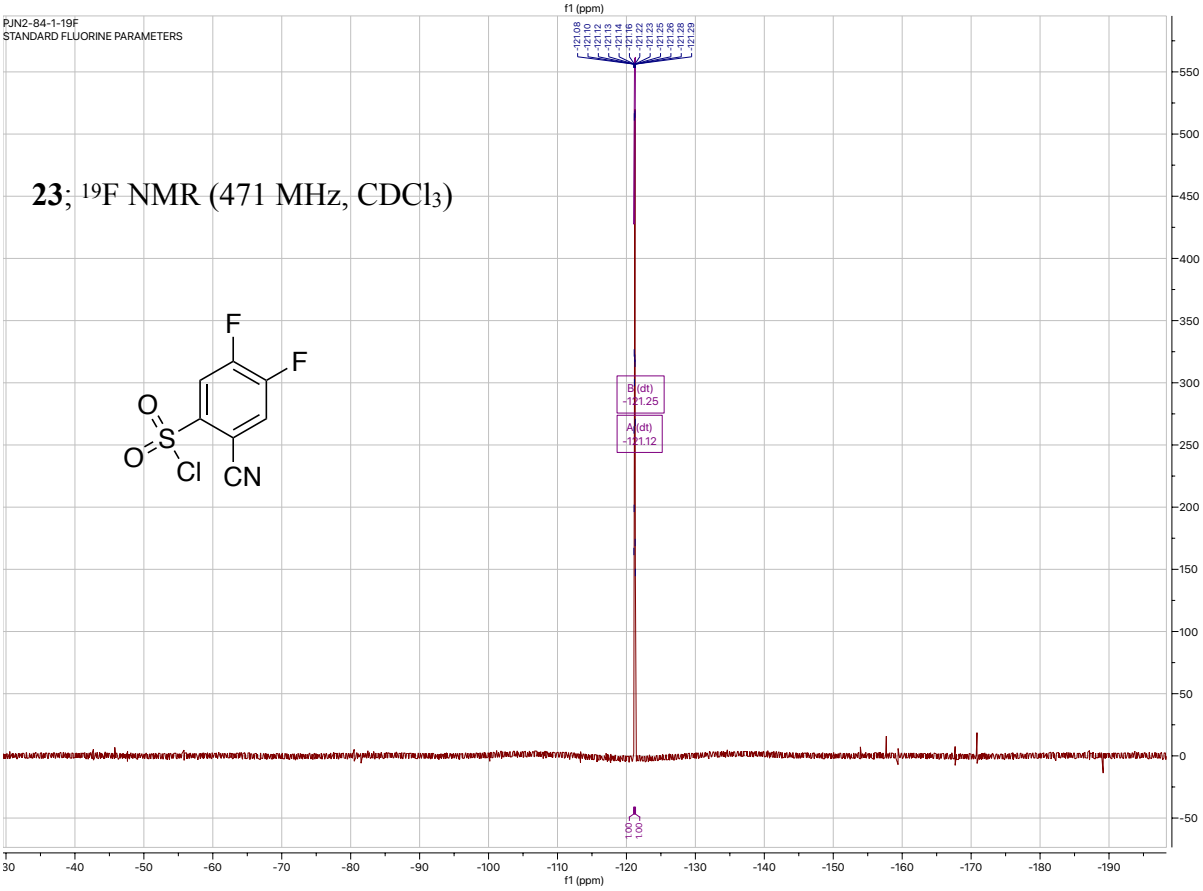

PJN2-83-1-1H
22; 1H NMR (500 MHz, CDCl3)
F
F
S
Bn
CN
B (dd)
7.46
A (dd)
7.17
C (m)
7.30
D (s)
4.20
f1 (ppm)

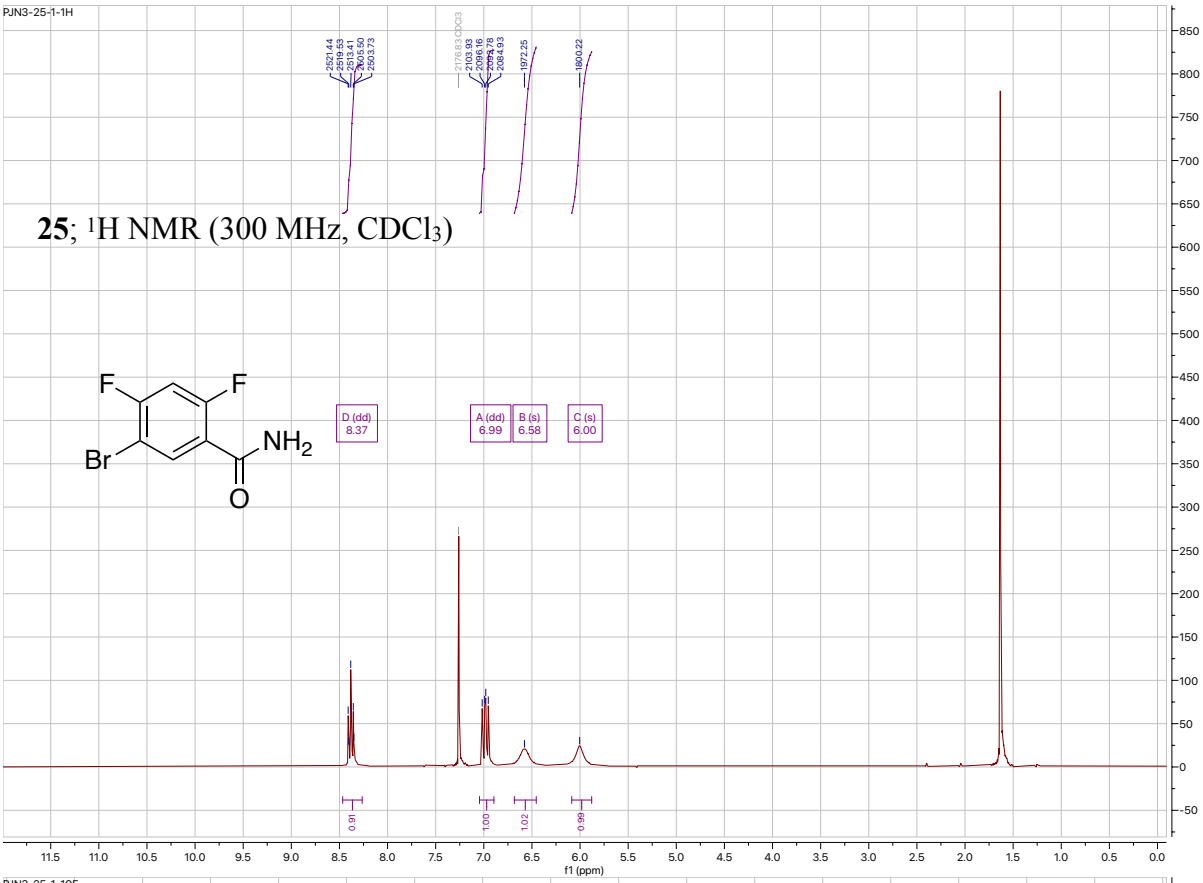

PJN2-83-1-19F
STANDARD FLUORINE PARAMETERS
22; 19F NMR (471 MHz, CDCl3)
F
F
S
Bn
CN
A (ddd)
-126.25
B (ddd)
-136.23
f1 (ppm)

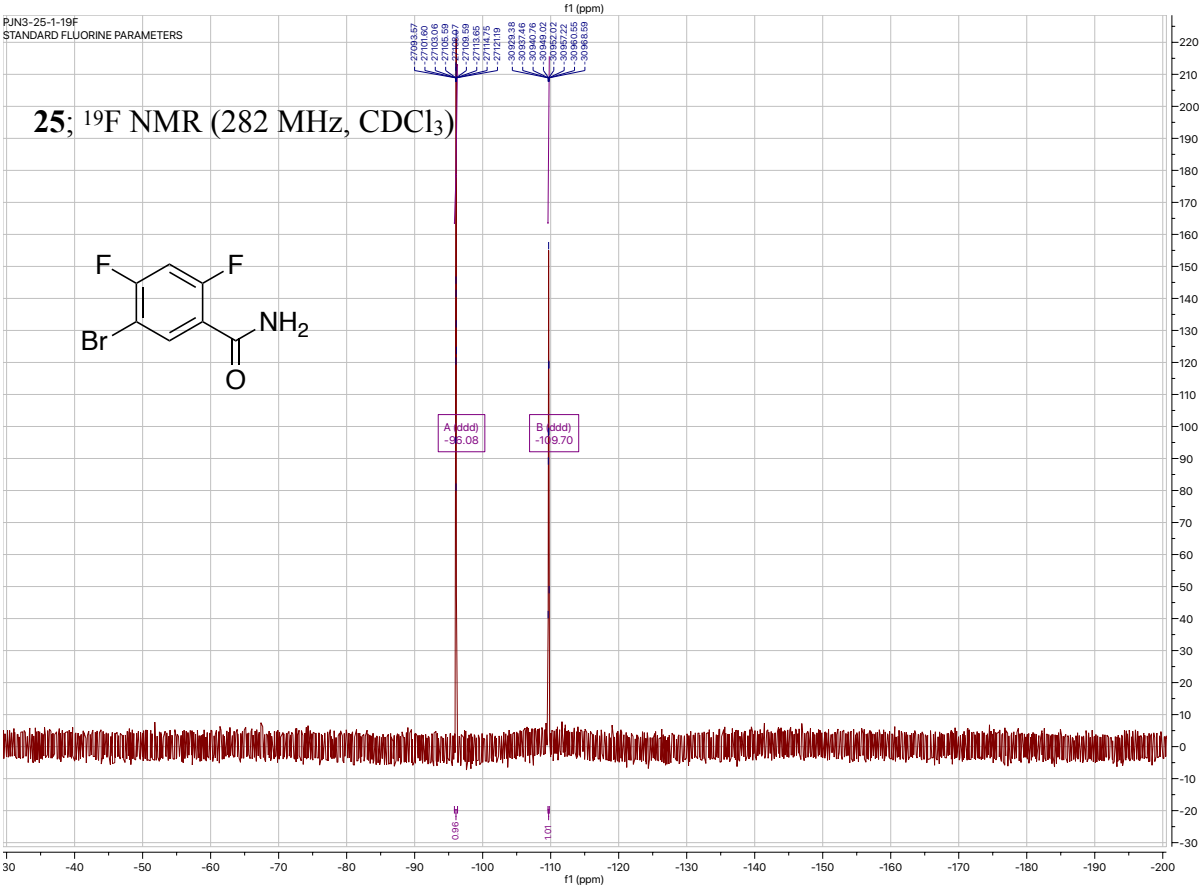
23; ^{1}H NMR (500 MHz, $CDCl_3$)

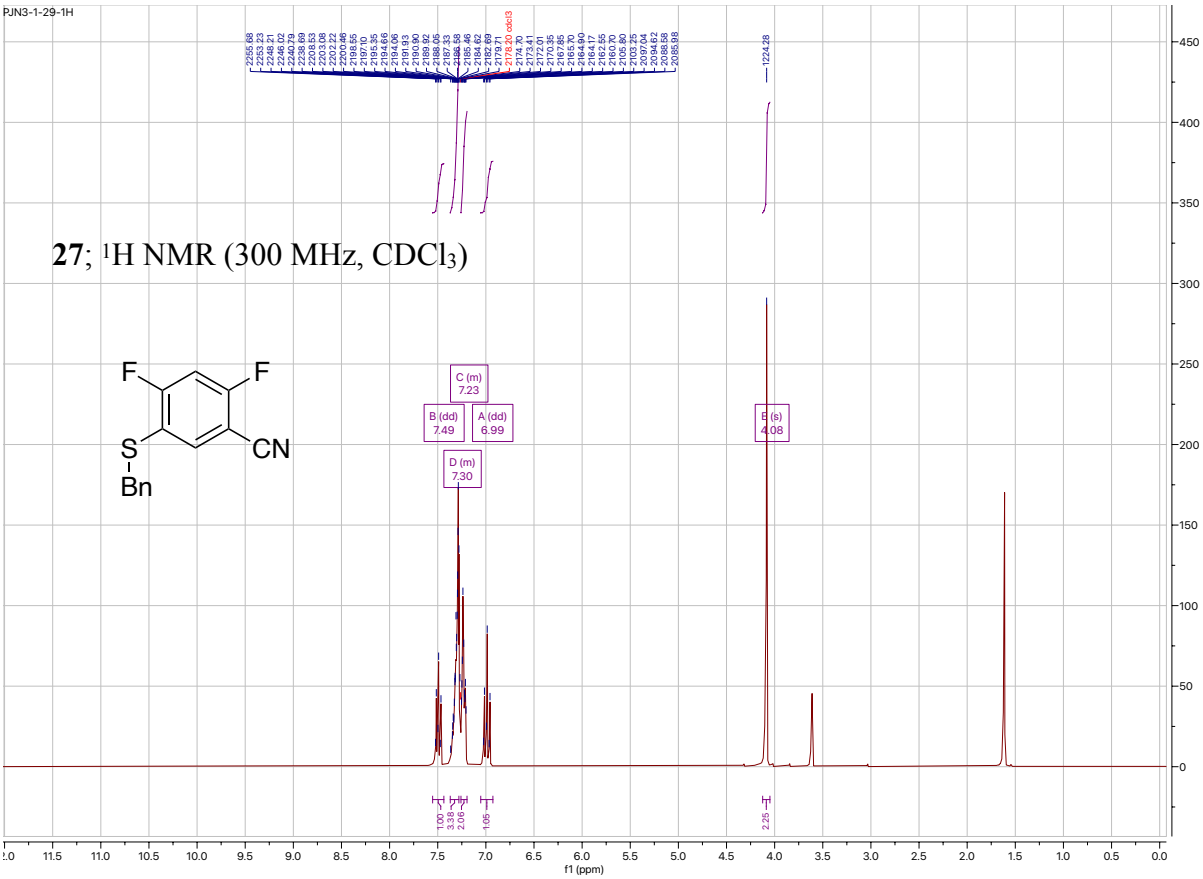


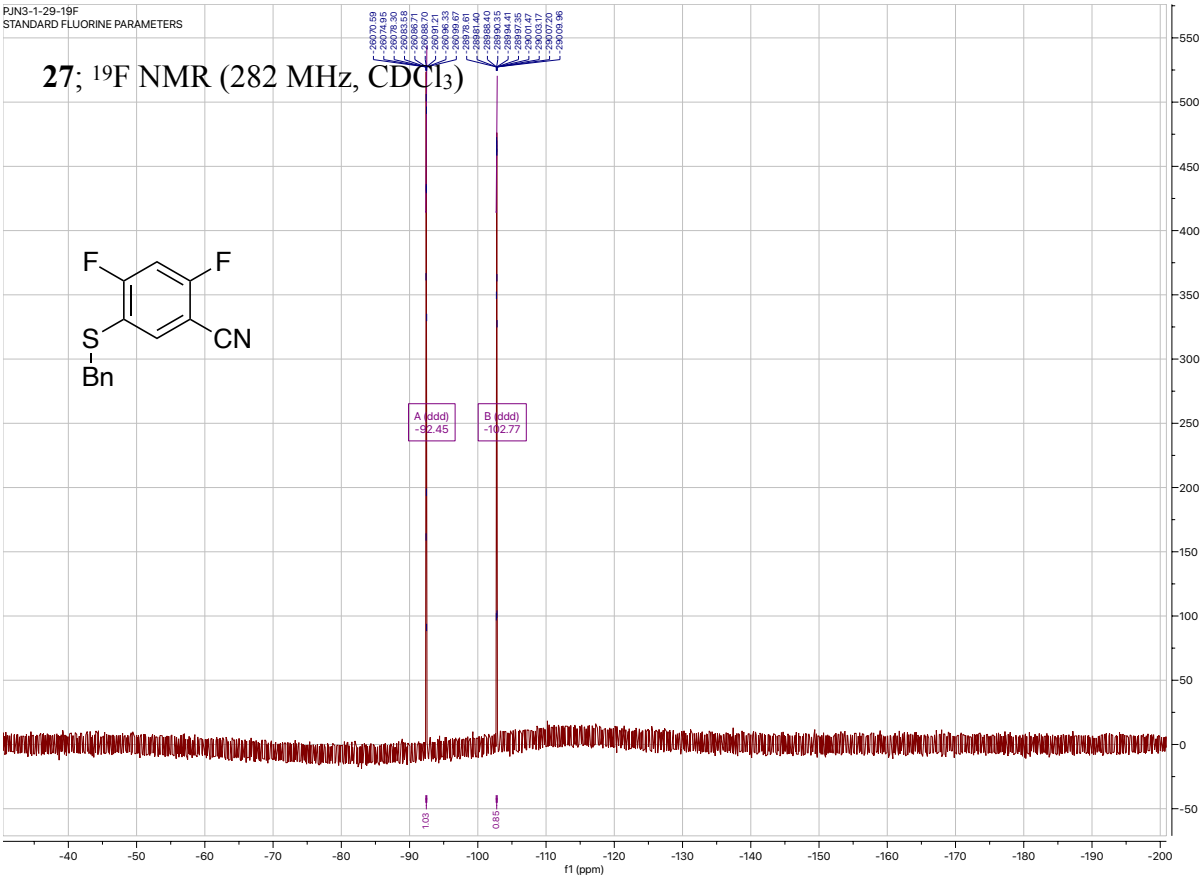
23; ^{19}F NMR (471 MHz, $CDCl_3$)

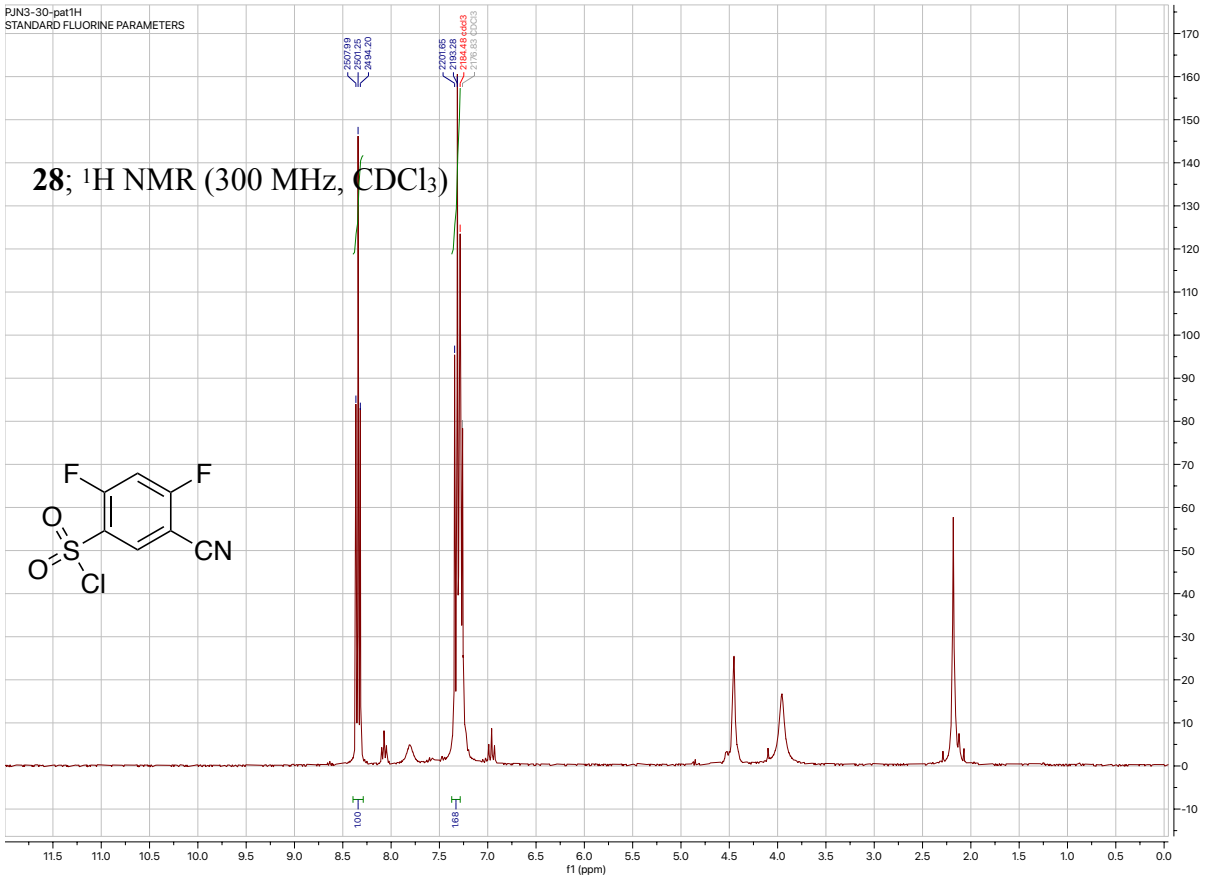


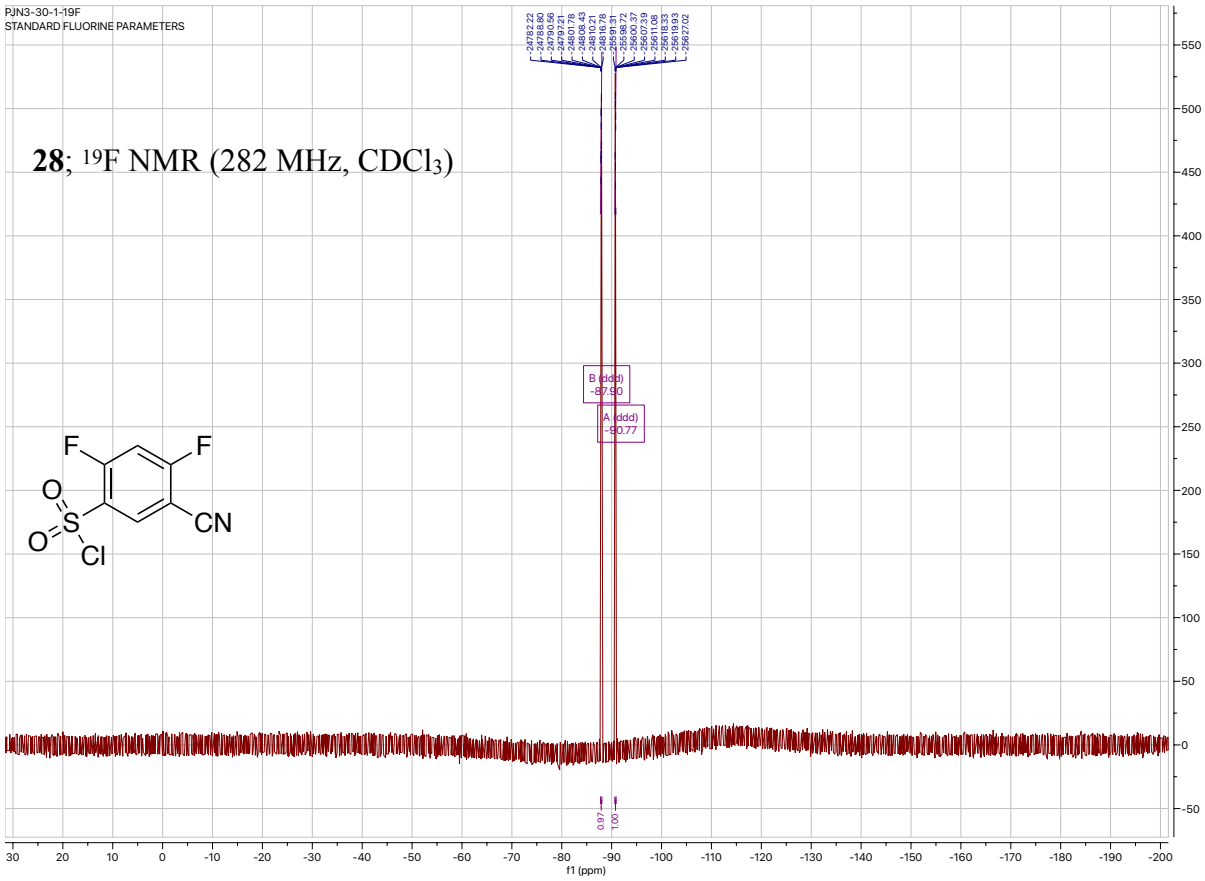

PJN3-25-1-1H
25; 1H NMR (300 MHz, CDCl3)
F
F
Br
NH2
O
D (dd) 8.37
A (dd) 6.99
B (s) 6.58
C (s) 6.00
f1 (ppm)

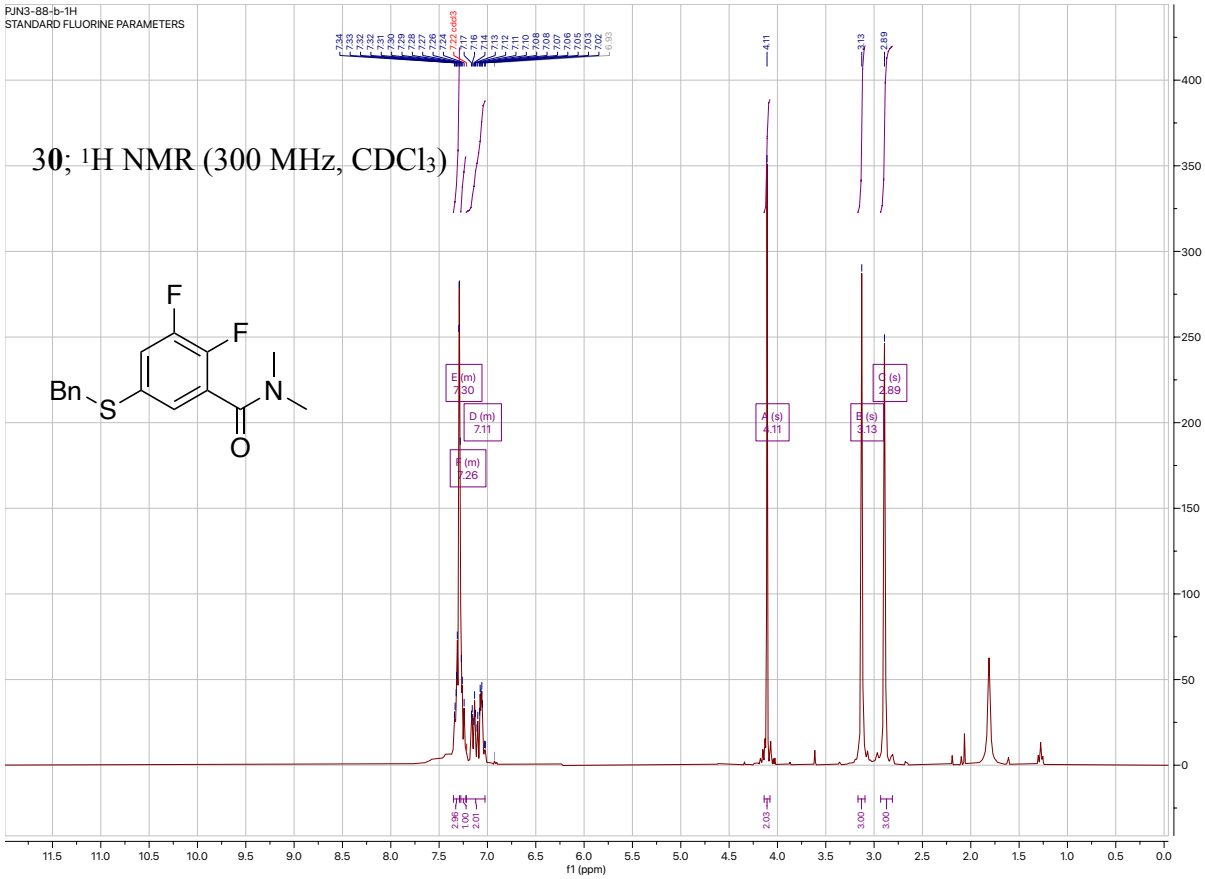

PJN3-25-1-19F
STANDARD FLUORINE PARAMETERS
25; 19F NMR (282 MHz, CDCl3)
F
F
Br
NH2
O
A (ddd) -96.08
B (ddd) -109.70
f1 (ppm)

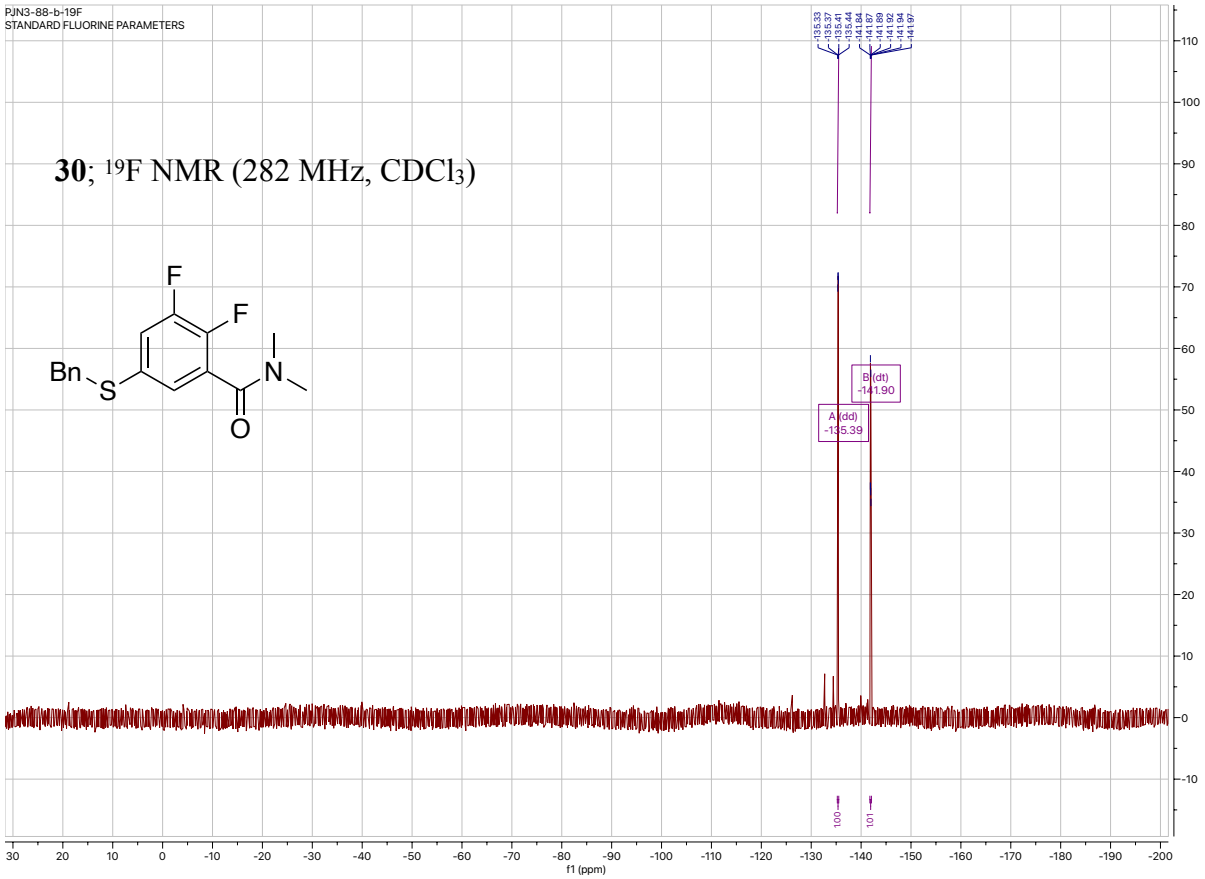

PJN3-1-29-1H
27; 1H NMR (300 MHz, CDCl3)
F
F
S
CN
Bn
C (m)
7.23
B (dd)
7.49
A (dd)
6.99
D (m)
7.30
E (s)
4.08
1.00
3.38
2.06
1.05
2.25
f1 (ppm)


PJN3-1-29-19F
STANDARD FLUORINE PARAMETERS
27; 19F NMR (282 MHz, CDCl3)
F
F
S
CN
Bn
A (ddd)
-92.45
B (ddd)
-102.77
1.03
0.85
f1 (ppm)

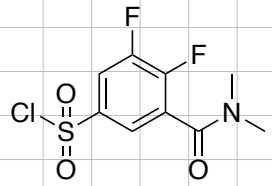

PJN3-30-pat1H
STANDARD FLUORINE PARAMETERS
28; 1H NMR (300 MHz, CDCl3)
F
F
O
S
O
Cl
CN
f1 (ppm)


PJN3-30-1-19F
STANDARD FLUORINE PARAMETERS
28; 19F NMR (282 MHz, CDCl3)
F
F
O
S
O
Cl
CN
B (ddd)
-87.90
A (ddd)
-90.77
f1 (ppm)

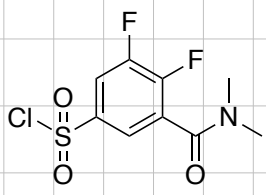

PJN3-88-b-1H
STANDARD FLUORINE PARAMETERS
30; 1H NMR (300 MHz, CDCl3)
F
F
Bn
S
N
O
E (m) 7.30
D (m) 7.11
F (m) 7.26
A (s) 4.11
B (s) 3.13
C (s) 2.89
f1 (ppm)

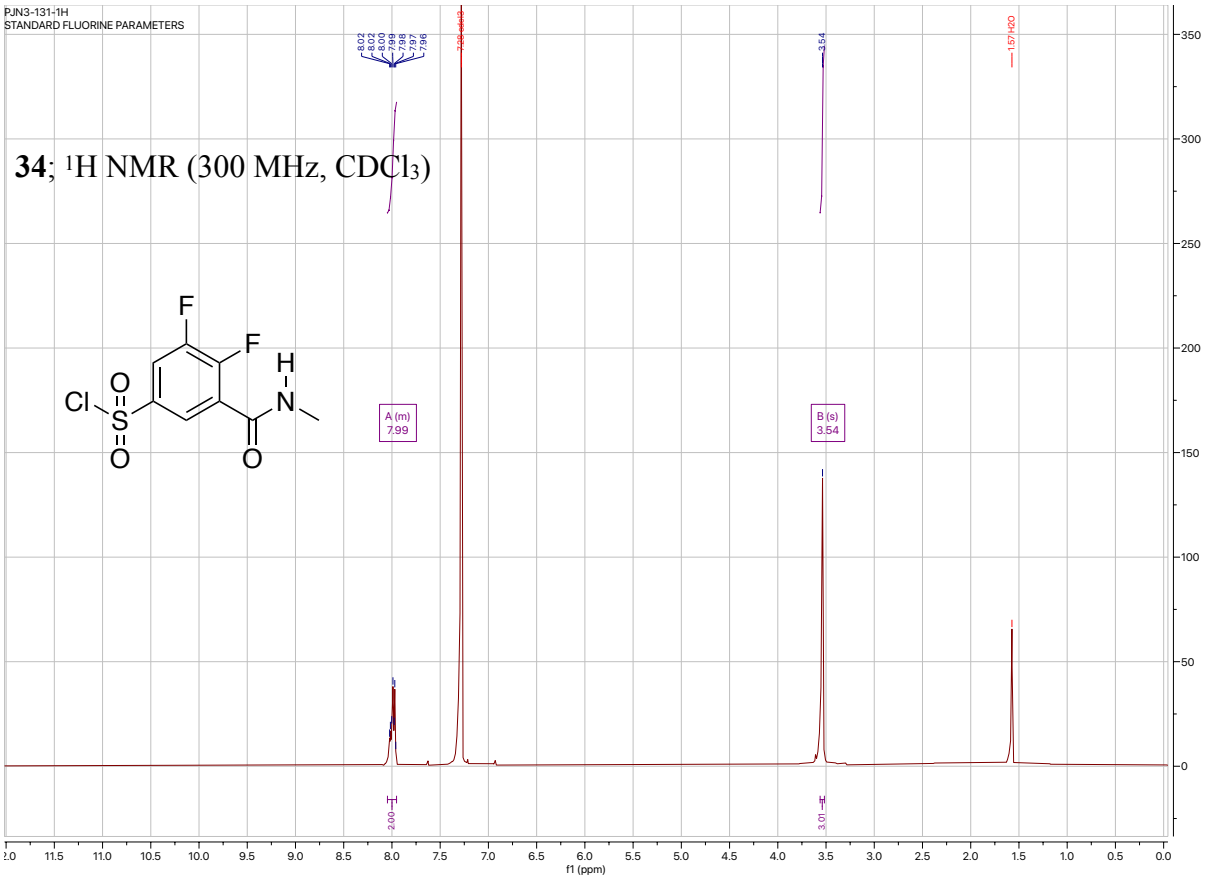

PJN3-88-b-19F
STANDARD FLUORINE PARAMETERS
30; 19F NMR (282 MHz, CDCl3)
F
F
Bn
S
N
O
B (dt) -141.90
A (dd) -135.39
f1 (ppm)

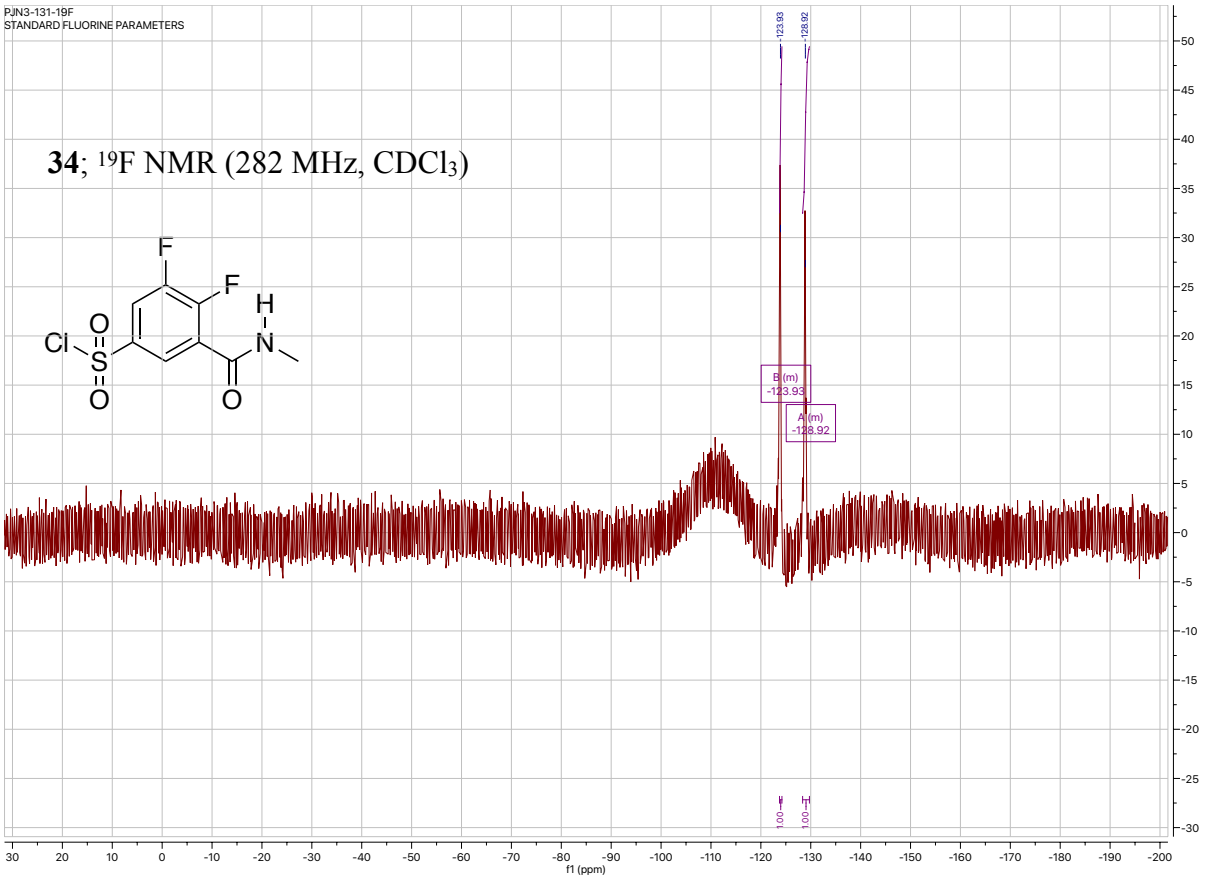
31; ^{1}H NMR (300 MHz, $CDCl_3$)

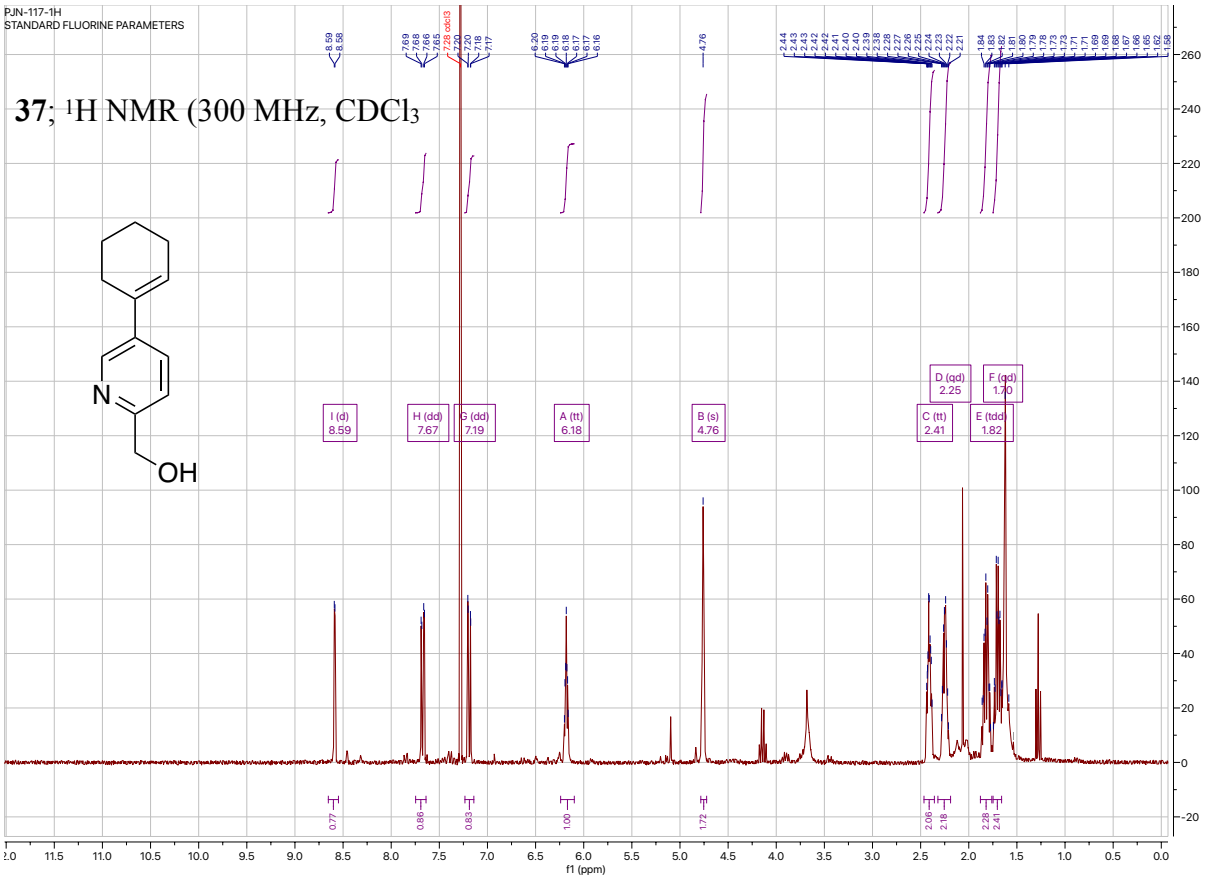


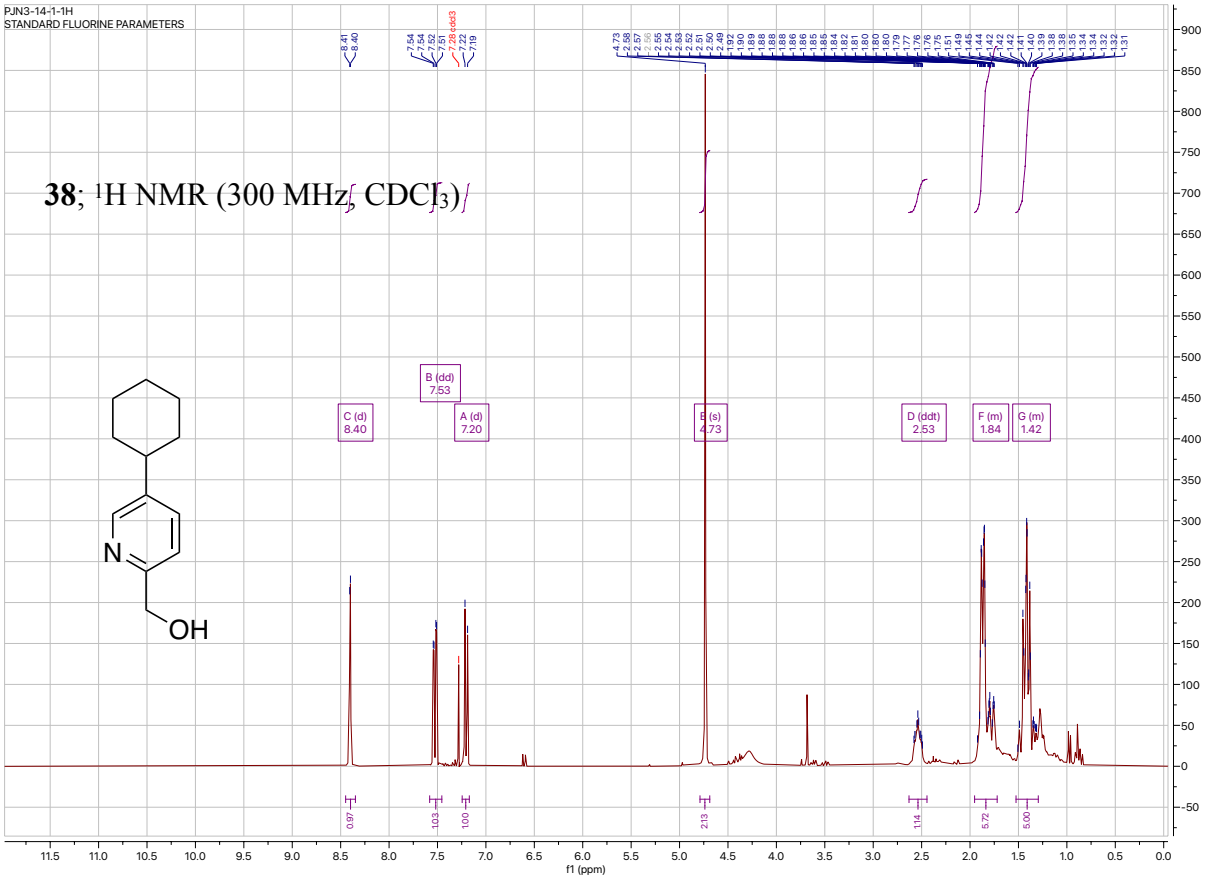
31; ^{19}F NMR (282 MHz, $CDCl_3$)

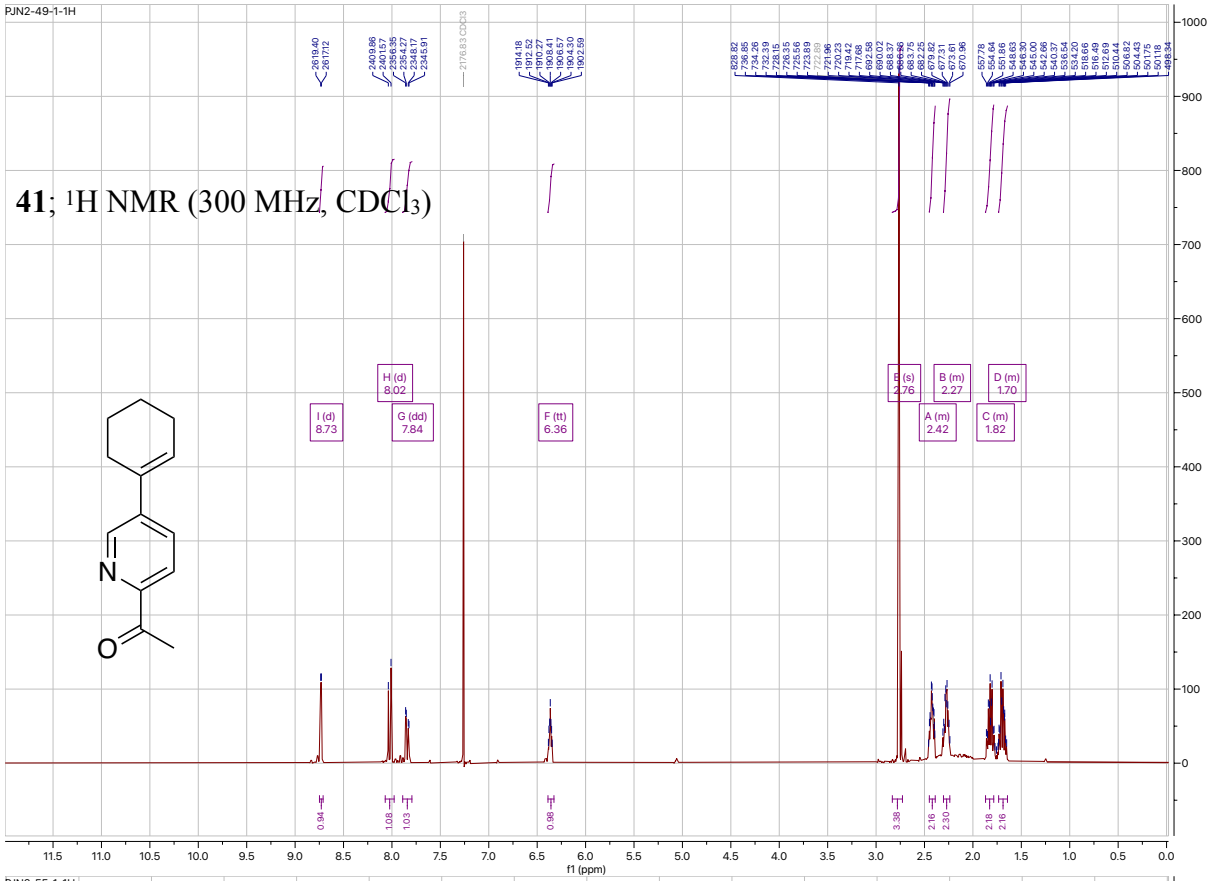


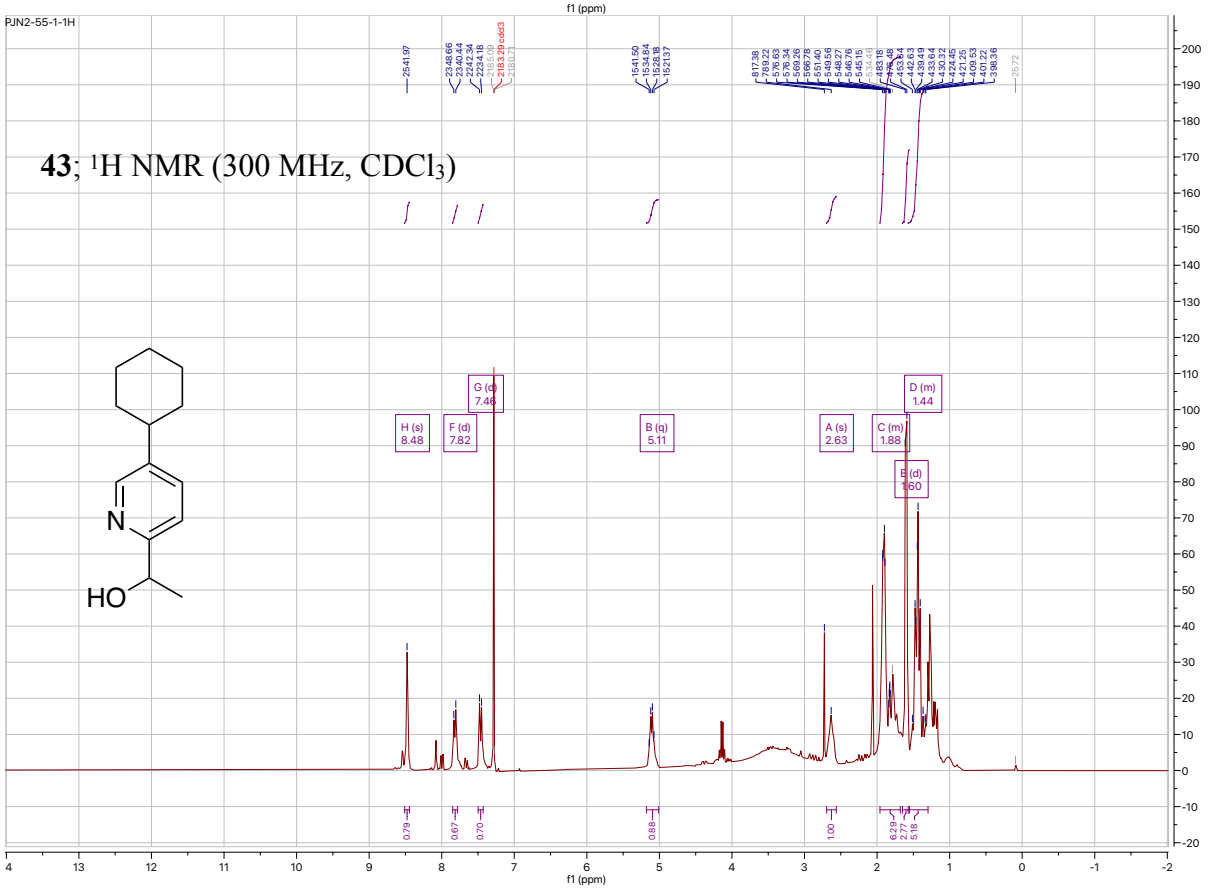

PJN3-131-1H
STANDARD FLUORINE PARAMETERS
34; 1H NMR (300 MHz, CDCl3)
F
F
H
N
Cl
O
S
O
O
A (m)
7.99
B (s)
3.54
1.57 H2O
f1 (ppm)

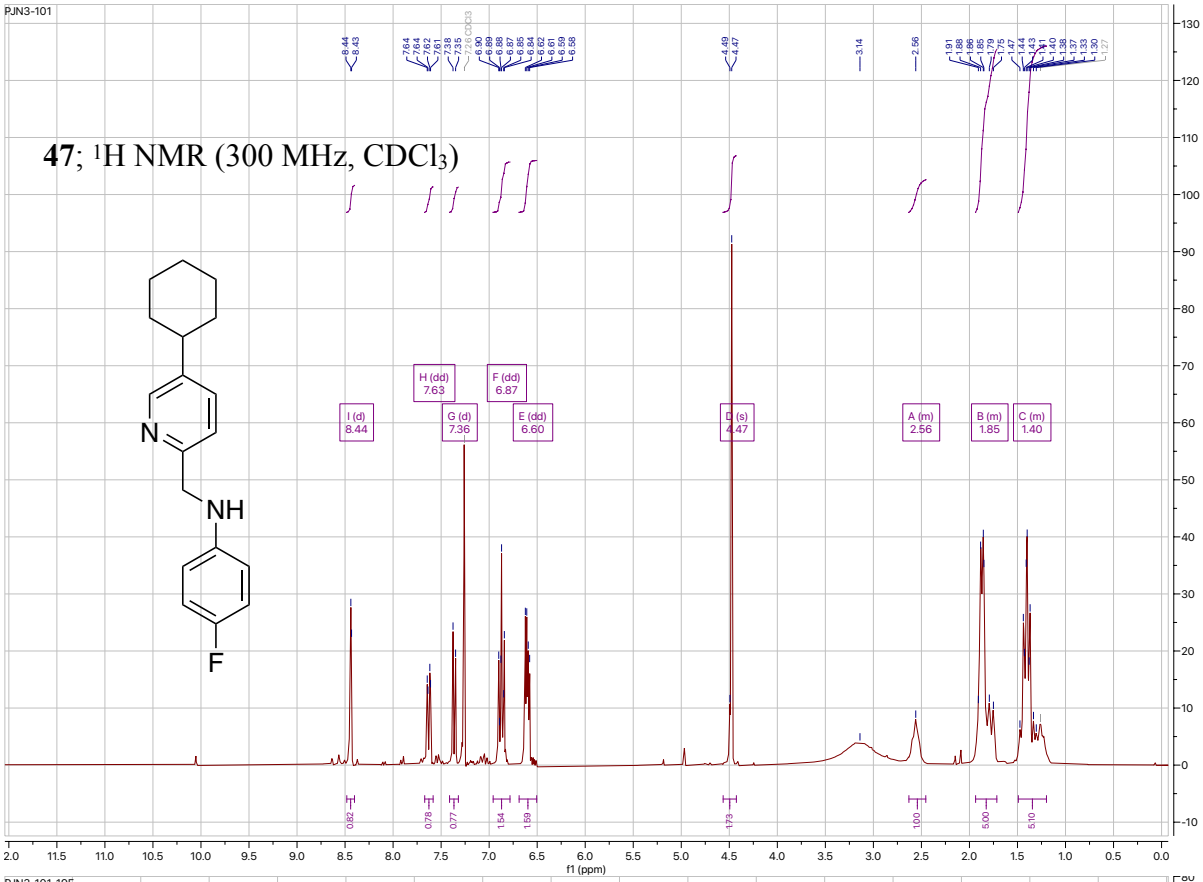

PJN3-131-19F
STANDARD FLUORINE PARAMETERS
34; 19F NMR (282 MHz, CDCl3)
F
F
H
N
Cl
O
S
O
O
B (m)
-123.93
A (m)
-128.92
f1 (ppm)

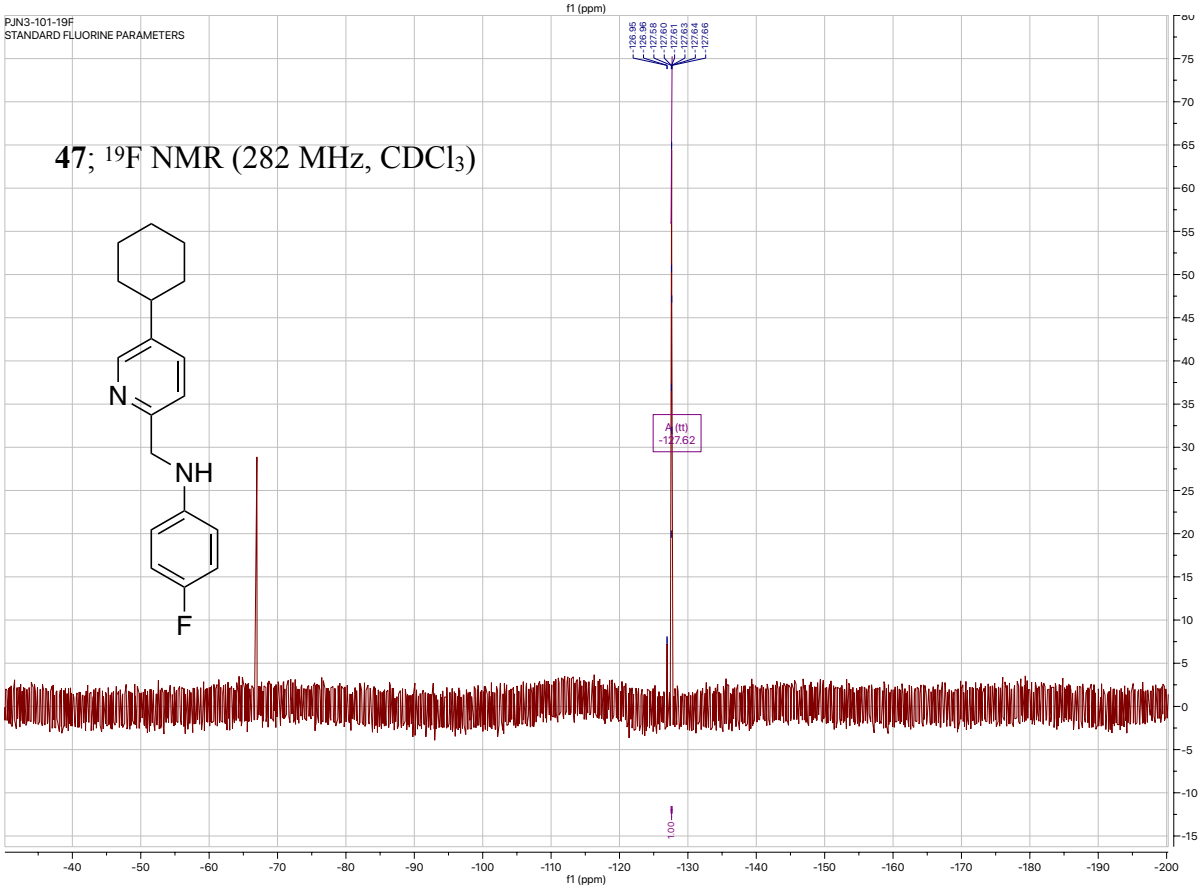

PJN-117-1H
STANDARD FLUORINE PARAMETERS
37; 1H NMR (300 MHz, CDCl3
N
OH
I (d) 8.59
H (dd) 7.67
G (dd) 7.19
A (tt) 6.18
B (s) 4.76
C (tt) 2.41
D (qd) 2.25
E (tdd) 1.82
F (qd) 1.70
f1 (ppm)

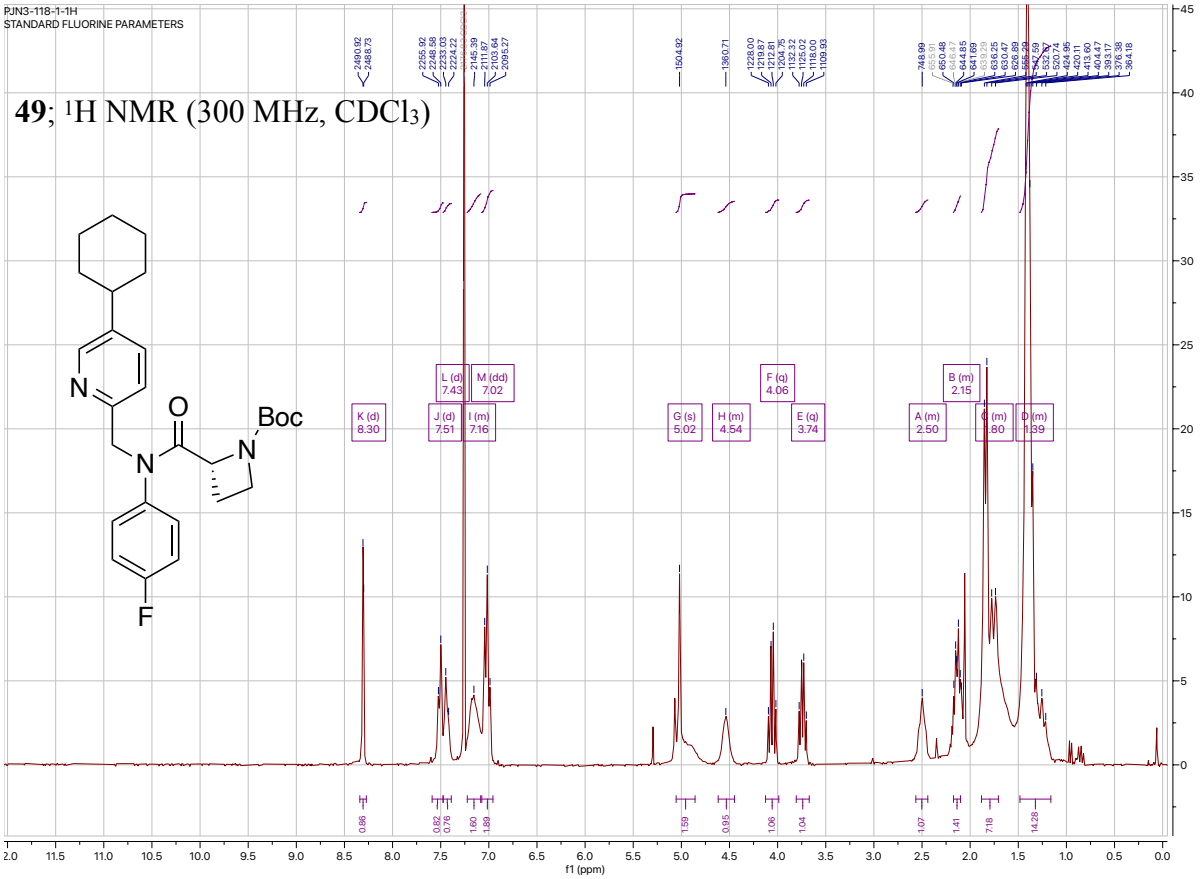

PJN3-14-1-1H
STANDARD FLUORINE PARAMETERS
38; 1H NMR (300 MHz, CDCl3)
N
OH
C (d) 8.40
B (dd) 7.53
A (d) 7.20
E (s) 4.73
D (ddt) 2.53
F (m) 1.84
G (m) 1.42
f1 (ppm)

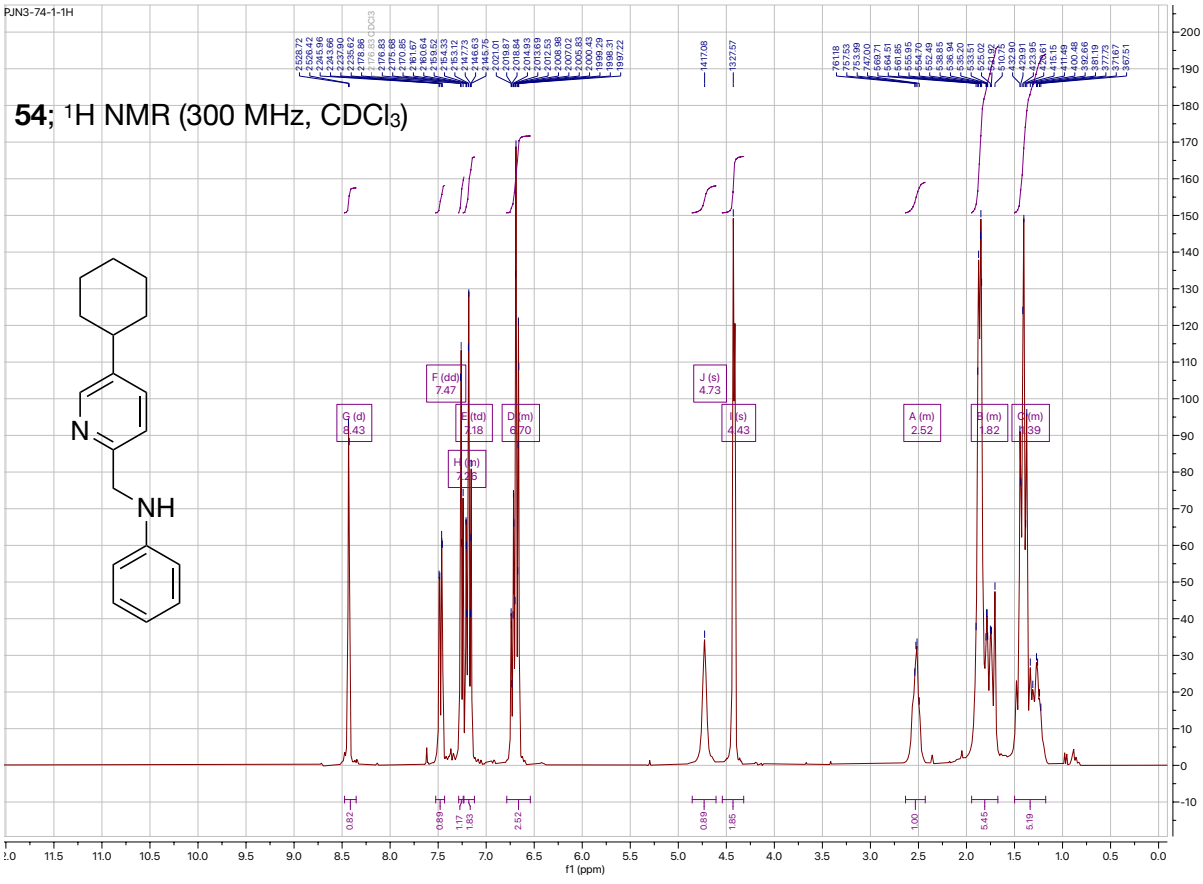

PJN2-49-1-1H
41; 1H NMR (300 MHz, CDCl3)
I (d) 8.73
H (d) 8.02
G (dd) 7.84
F (tt) 6.36
E (s) 2.76
B (m) 2.27
A (m) 2.42
D (m) 1.70
C (m) 1.82
N
O
f1 (ppm)

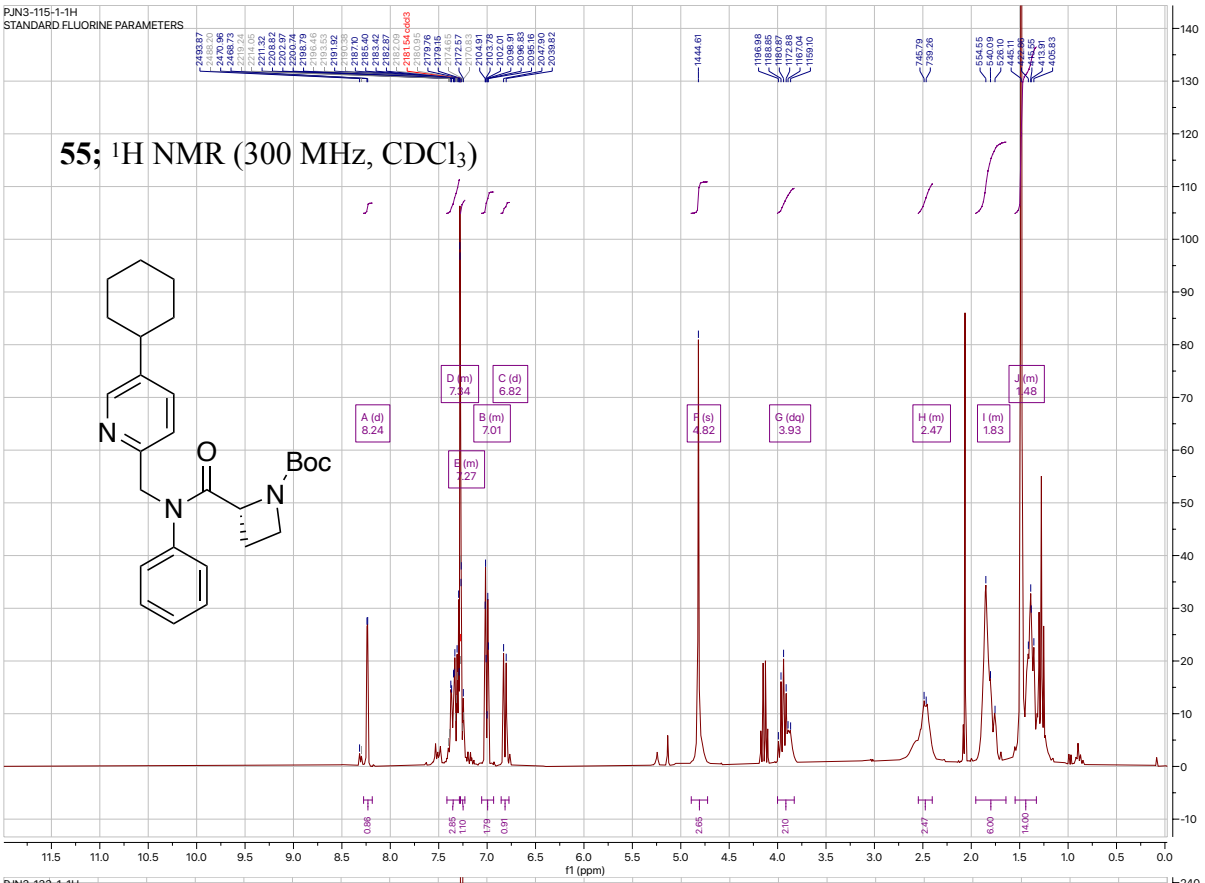

PJN2-55-1-1H
43; 1H NMR (300 MHz, CDCl3)
H (s) 8.48
F (d) 7.82
G (d) 7.46
B (q) 5.11
A (s) 2.63
C (m) 1.88
D (m) 1.44
E (d) 1.60
N
HO
f1 (ppm)

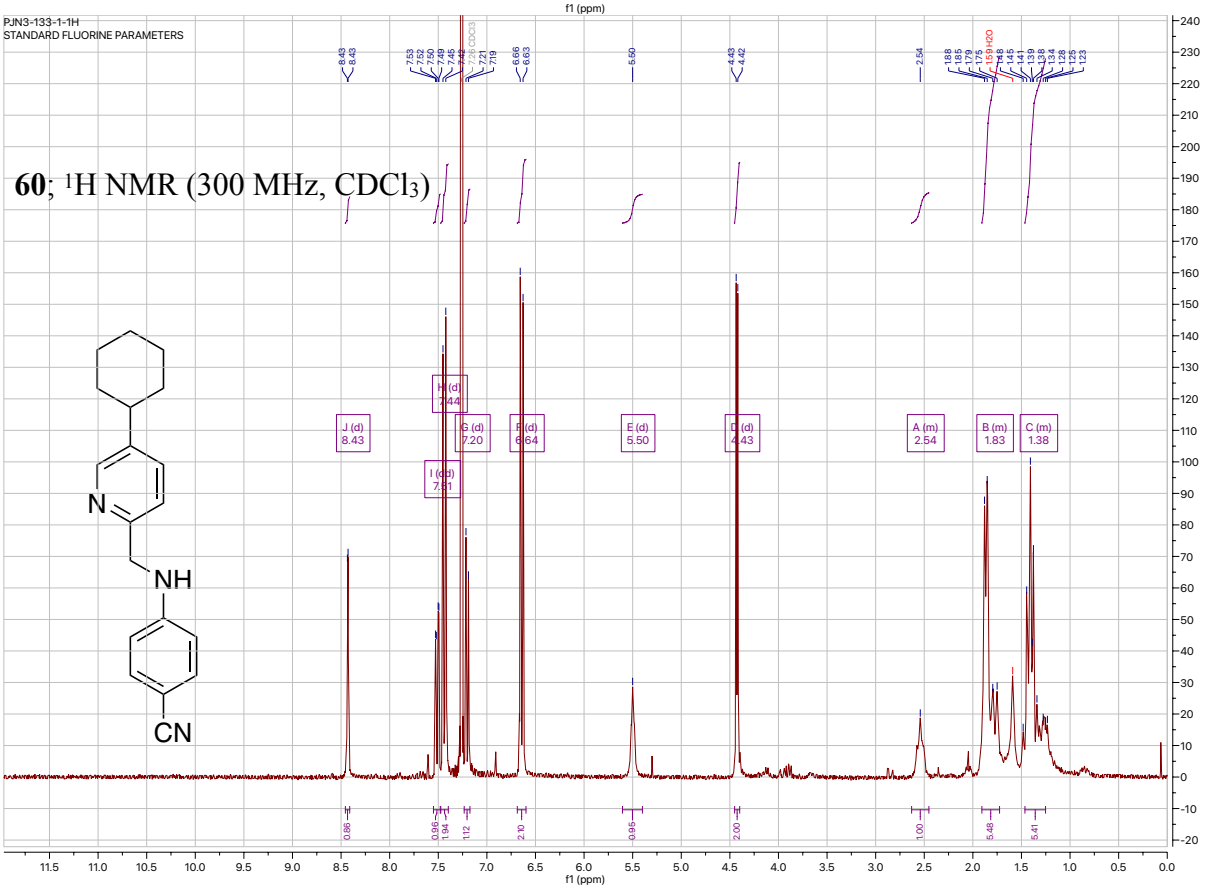

PJN3-101
47; 1H NMR (300 MHz, CDCl3)
H (dd) 7.63
F (dd) 6.87
I (d) 8.44
G (d) 7.36
E (dd) 6.60
D (s) 4.47
A (m) 2.56
B (m) 1.85
C (m) 1.40
N
NH
F
f1 (ppm)

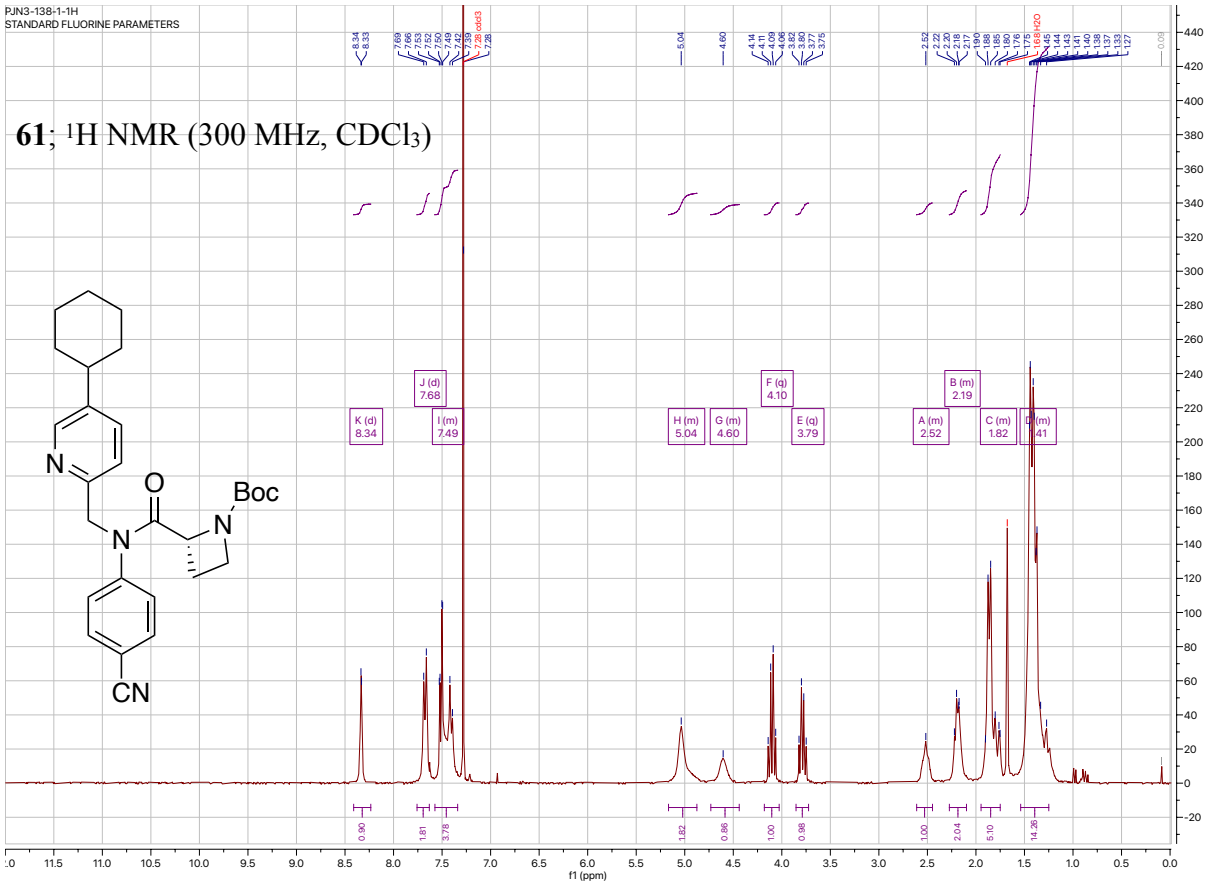

PJN3-101-19F
STANDARD FLUORINE PARAMETERS
47; 19F NMR (282 MHz, CDCl3)
A (tt) -127.62
N
NH
F
f1 (ppm)

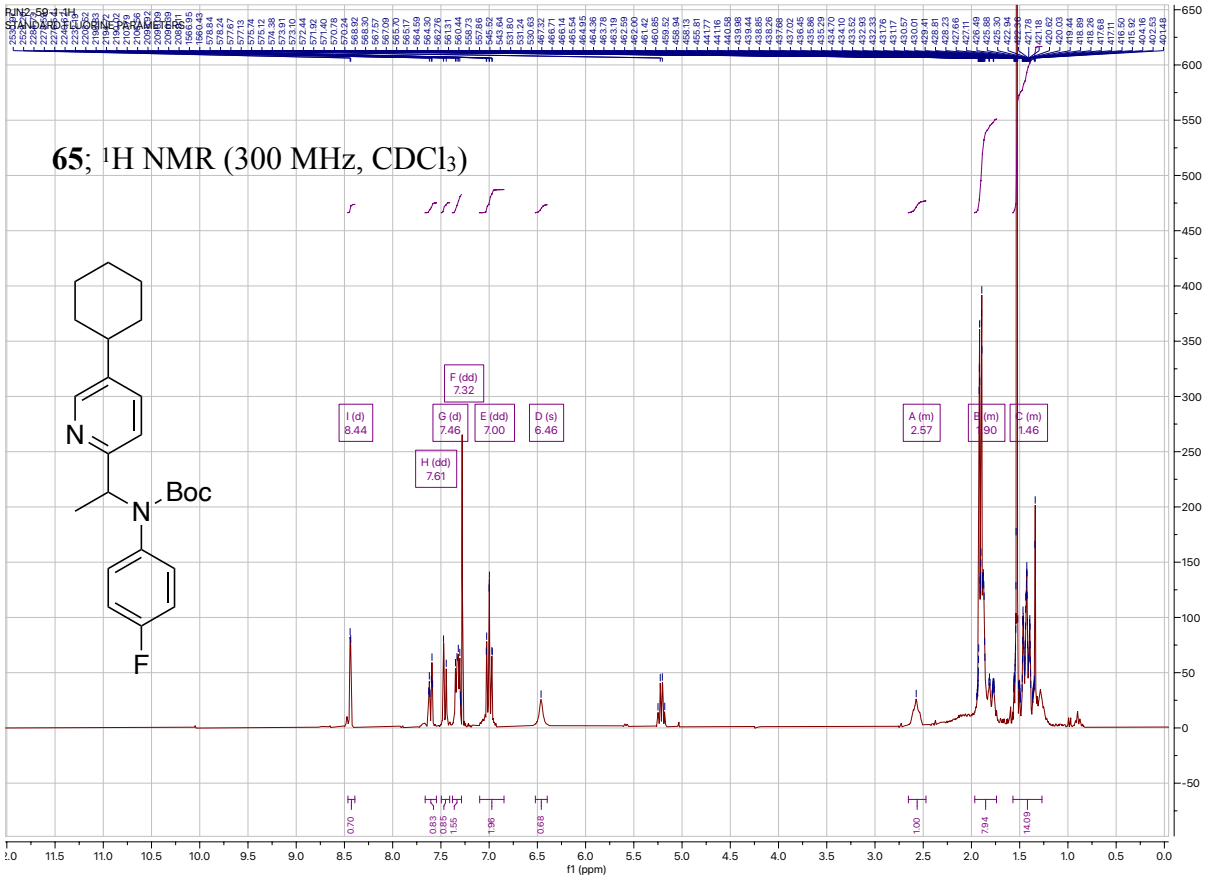

PJN3-118-1-1H
STANDARD FLUORINE PARAMETERS
49; 1H NMR (300 MHz, CDCl3)
Boc
F
K (d) 8.30
L (d) 7.43
M (dd) 7.02
J (d) 7.51
I (m) 7.16
G (s) 5.02
H (m) 4.54
F (q) 4.06
E (q) 3.74
A (m) 2.50
B (m) 2.15
C (m) 1.80
D (m) 1.39
f1 (ppm)

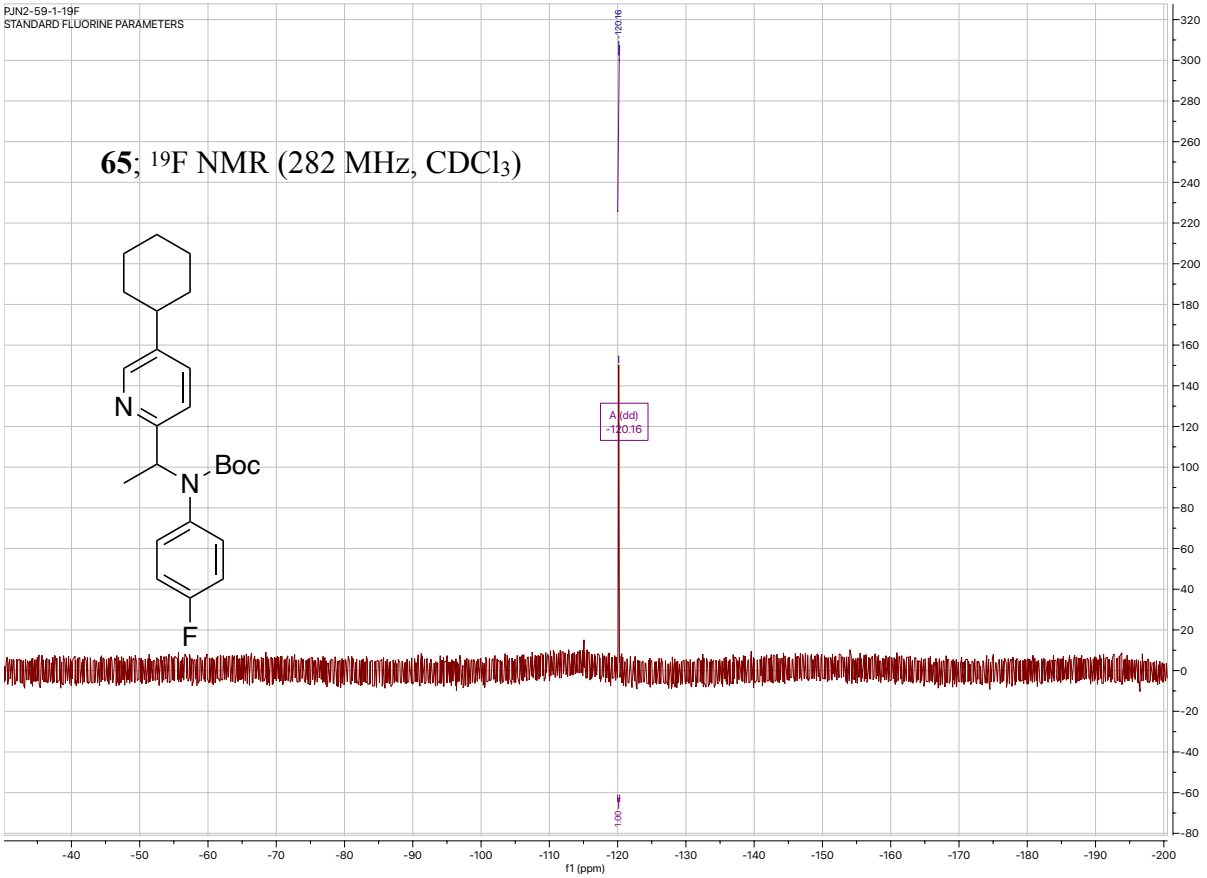

PJN3-74-1-1H
54; 1H NMR (300 MHz, CDCl3)
NH
G (d) 8.43
F (dd) 7.47
E (td) 7.18
D (m) 6.70
H (m) 2.58
J (s) 4.73
I (s) 4.43
A (m) 2.52
B (m) 1.82
C (m) 1.39
f1 (ppm)

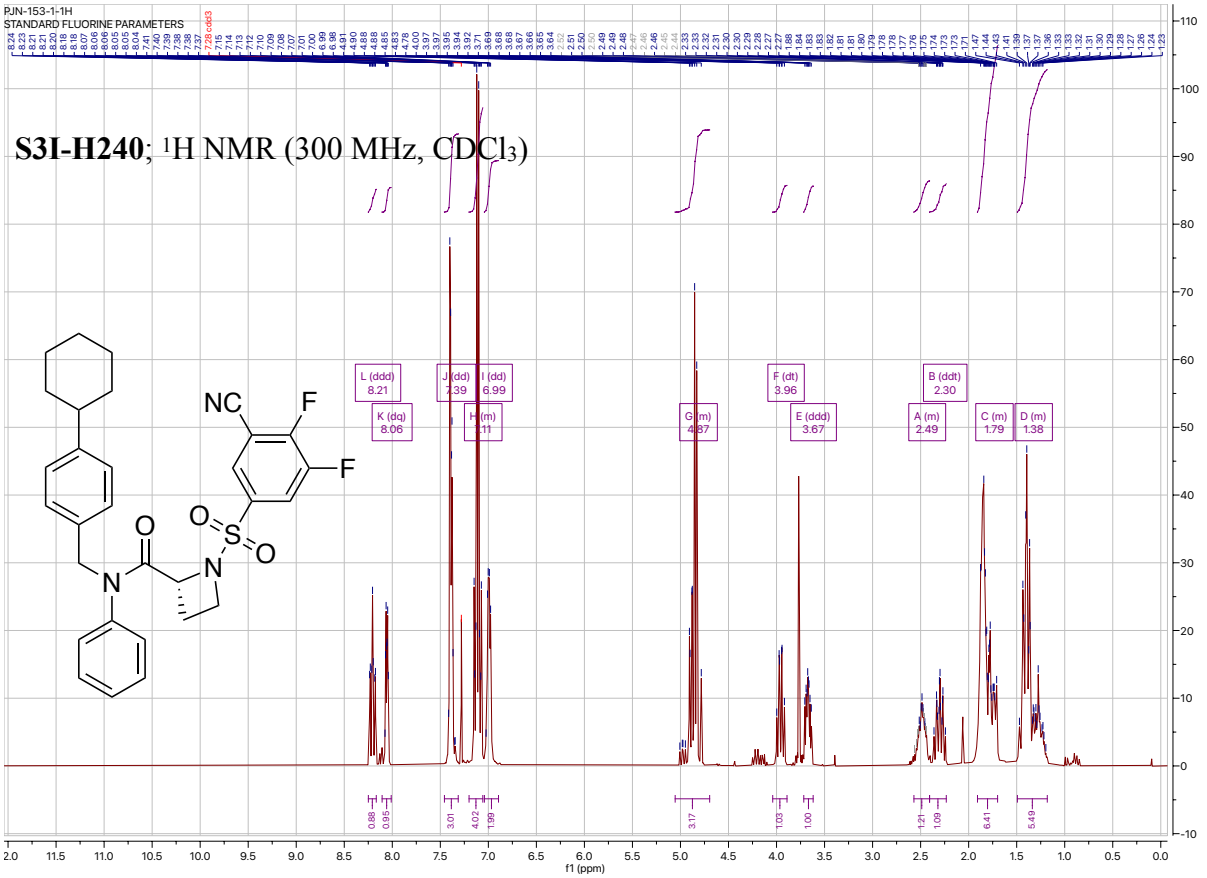

PJN3-115i-1-1H
STANDARD FLUORINE PARAMETERS
55; 1H NMR (300 MHz, CDCl3)
Boc
A (d) 8.24
B (m) 7.01
C (d) 6.82
D (m) 7.34
E (m) 2.27
F (s) 4.82
G (dq) 3.93
H (m) 2.47
I (m) 1.83
J (m) 1.48
f1 (ppm)

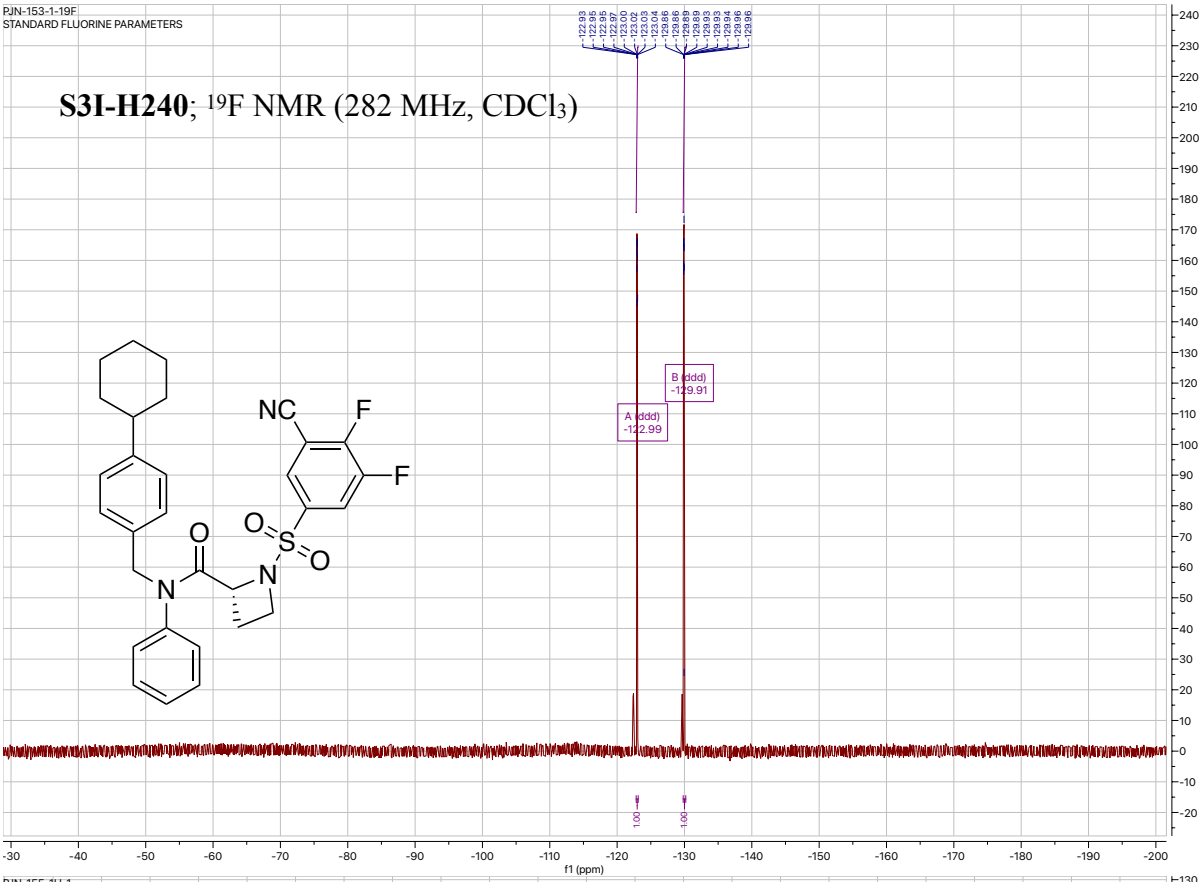

PJN3-133-1-1H
STANDARD FLUORINE PARAMETERS
60; 1H NMR (300 MHz, CDCl3)
NH
CN
J (d) 8.43
H (d) 7.44
G (d) 7.20
I (dd) 7.81
F (d) 6.64
E (d) 5.50
D (d) 4.43
A (m) 2.54
B (m) 1.83
C (m) 1.38
f1 (ppm)

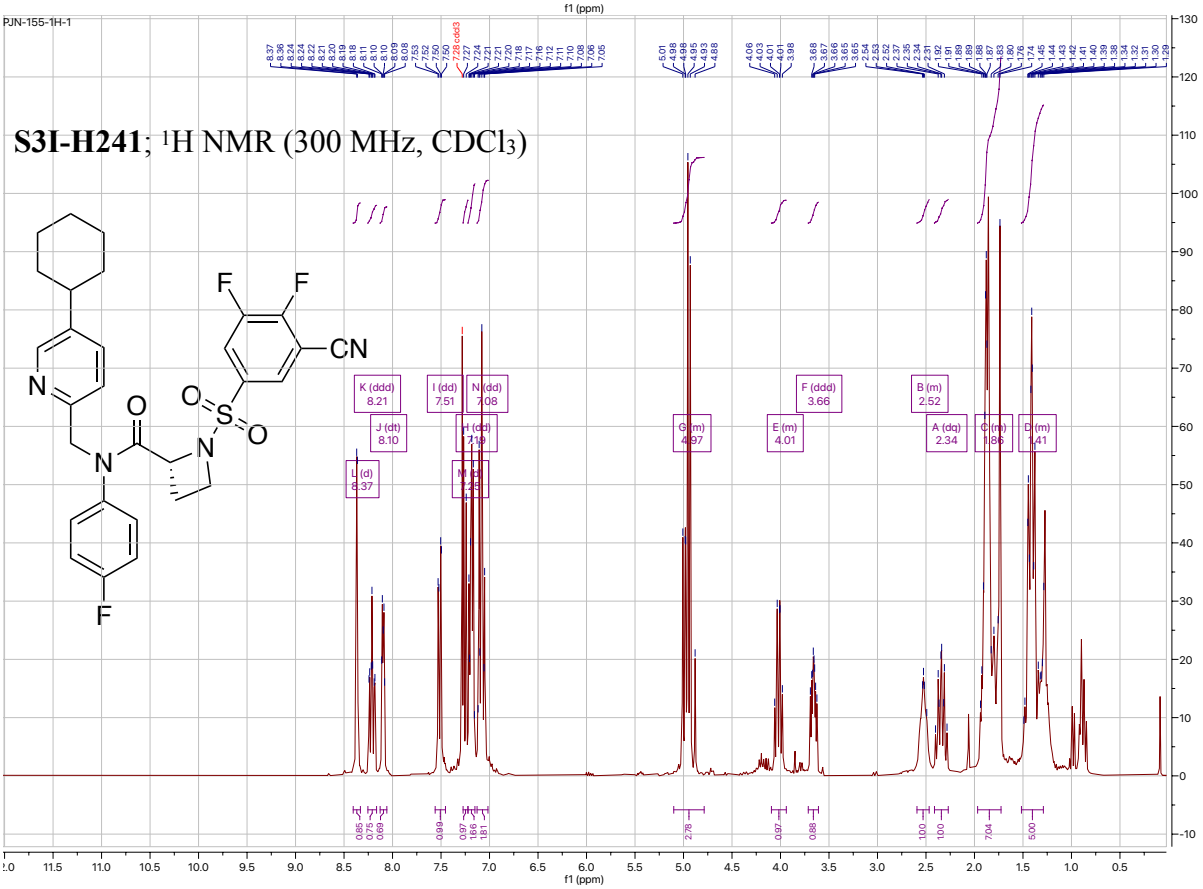

PJN3-138-1-1H
STANDARD FLUORINE PARAMETERS
61; 1H NMR (300 MHz, CDCl3)
K (d) 8.34
J (d) 7.68
I (m) 7.49
H (m) 5.04
G (m) 4.60
F (q) 4.10
E (q) 3.79
A (m) 2.52
B (m) 2.19
C (m) 1.82
D (m) 1.41
Boc
CN
f1 (ppm)

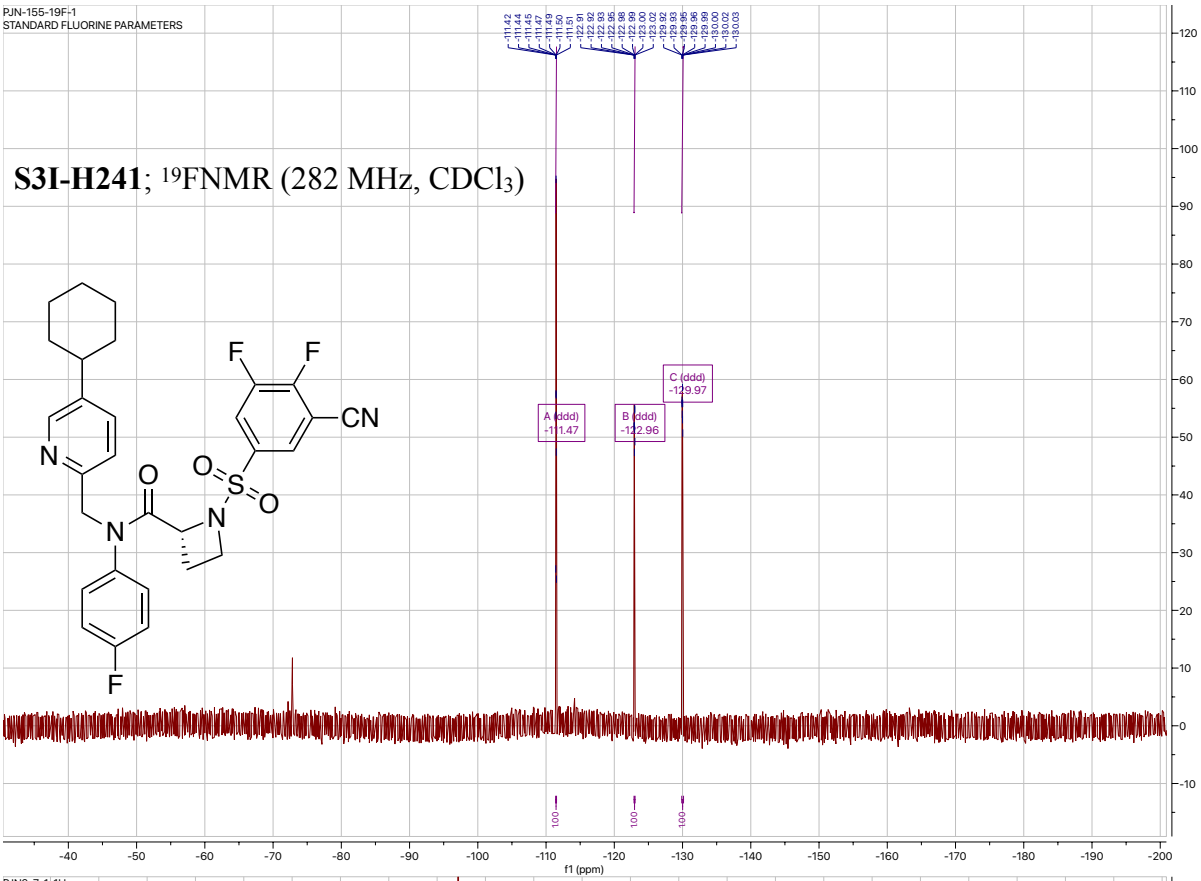

65; 1H NMR (300 MHz, CDCl3)
I (d) 8.44
F (dd) 7.32
G (d) 7.46
E (dd) 7.00
D (s) 6.46
H (dd) 7.61
A (m) 2.57
B (m) 1.90
C (m) 1.46
Boc
F
f1 (ppm)


PJN2-59-1-19F
STANDARD FLUORINE PARAMETERS
65; 19F NMR (282 MHz, CDCl3)
N
Boc
N
F
A (dd)
-120.16
f1 (ppm)

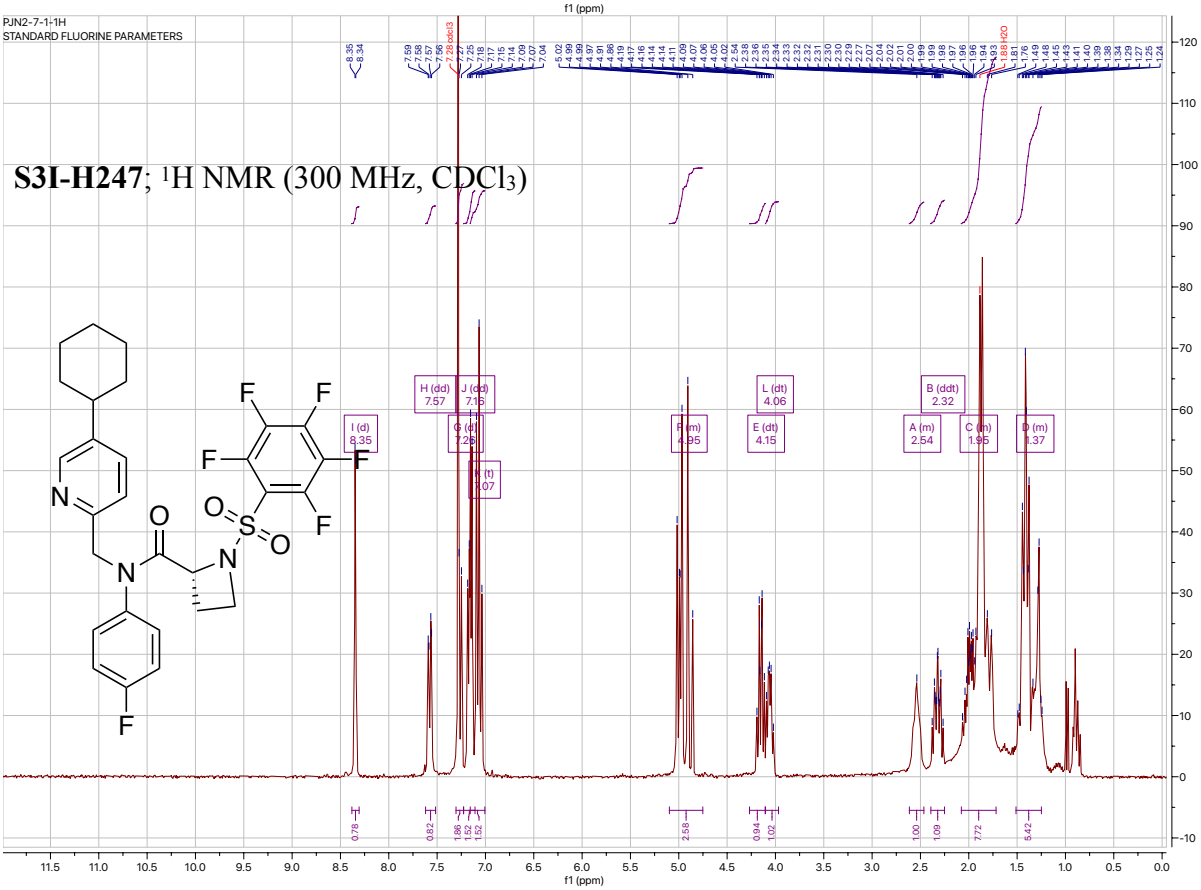

PJN-153-1-1H
STANDARD FLUORINE PARAMETERS
S3I-H240; 1H NMR (300 MHz, CDCl3)
NC
F
F
O
S
O
N
O
N
L (ddd)
8.21
K (dq)
8.06
J (dd)
7.39
I (dd)
6.99
H (m)
7.11
G (m)
4.87
F (dt)
3.96
E (ddd)
3.67
B (ddt)
2.30
A (m)
2.49
C (m)
1.79
D (m)
1.38
f1 (ppm)


PJN-153-1-19F
STANDARD FLUORINE PARAMETERS
S3I-H240; 19F NMR (282 MHz, CDCl3)
A (ddd)
-122.99
B (ddd)
-129.91
f1 (ppm)

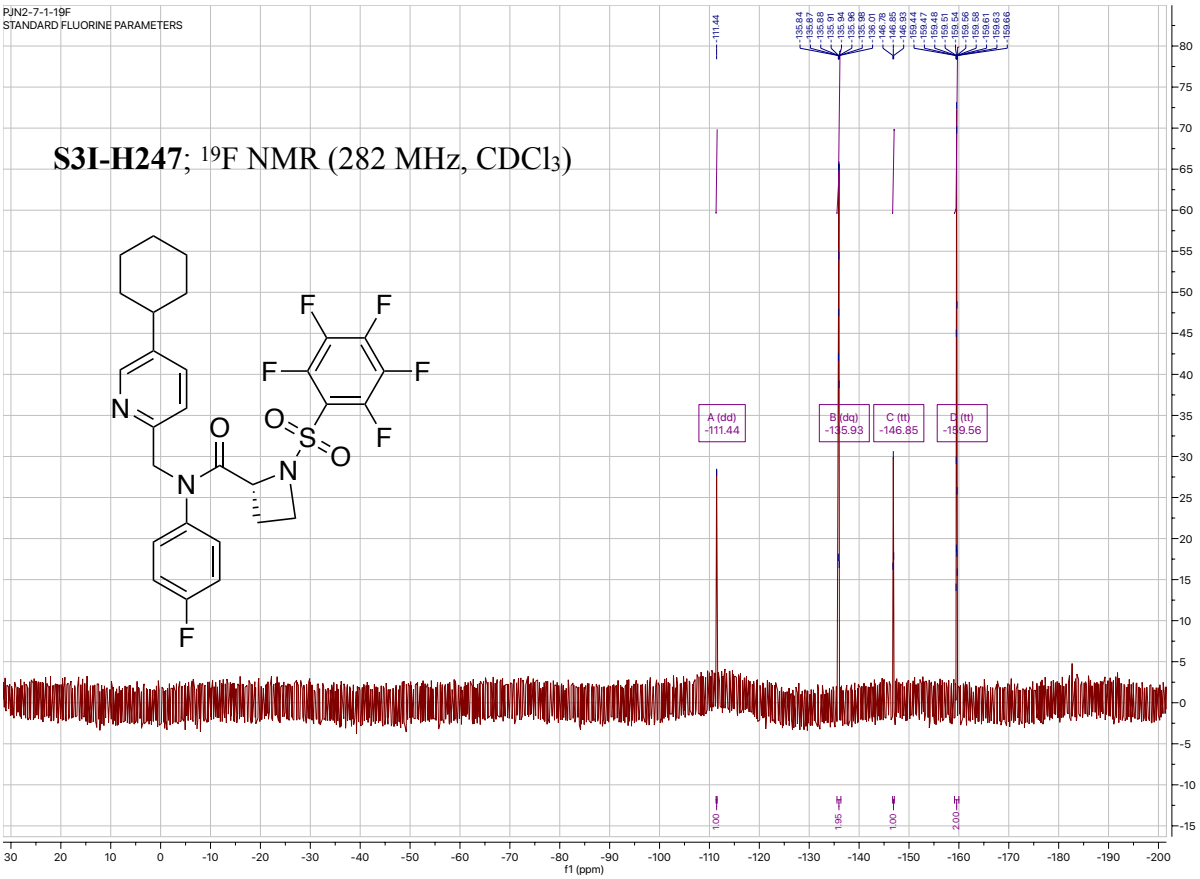

PJN-155-1H-1
S3I-H241; 1H NMR (300 MHz, CDCl3)
K (ddd)
8.21
I (dd)
7.51
N (dd)
7.08
J (dt)
8.10
H (dd)
7.19
L (d)
8.37
G (m)
4.97
F (ddd)
3.66
E (m)
4.01
B (m)
2.52
A (dq)
2.34
C (m)
1.86
D (m)
1.41
f1 (ppm)

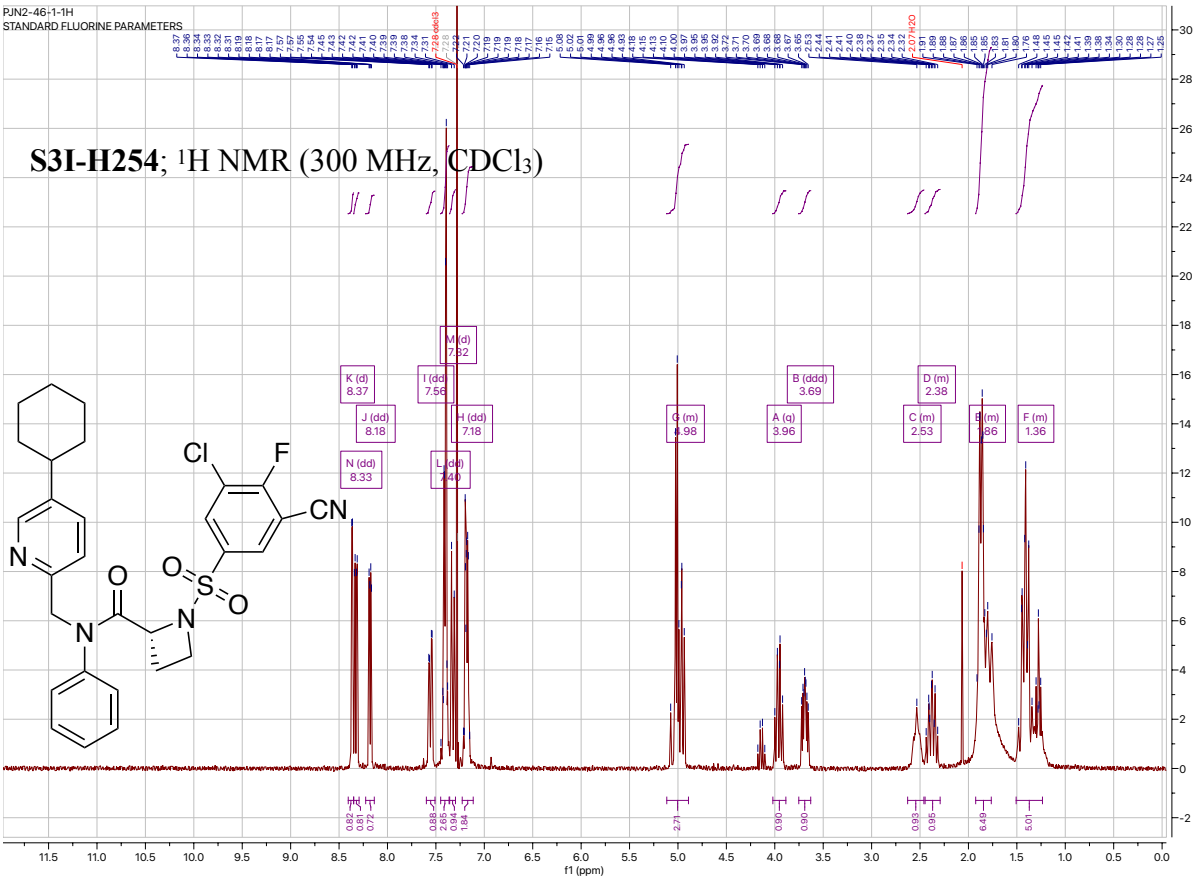


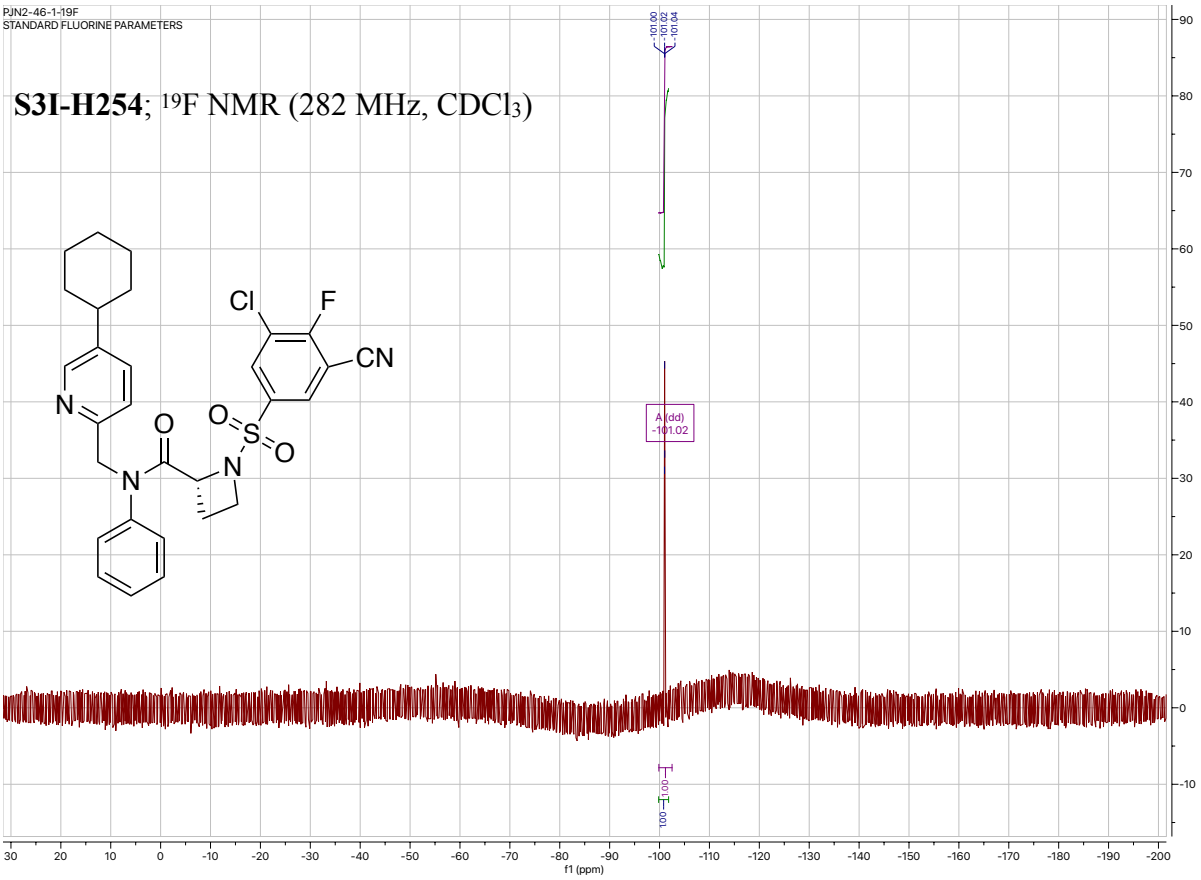
S3I-H241; ^{19}FNMR (282 MHz, $CDCl_3$)

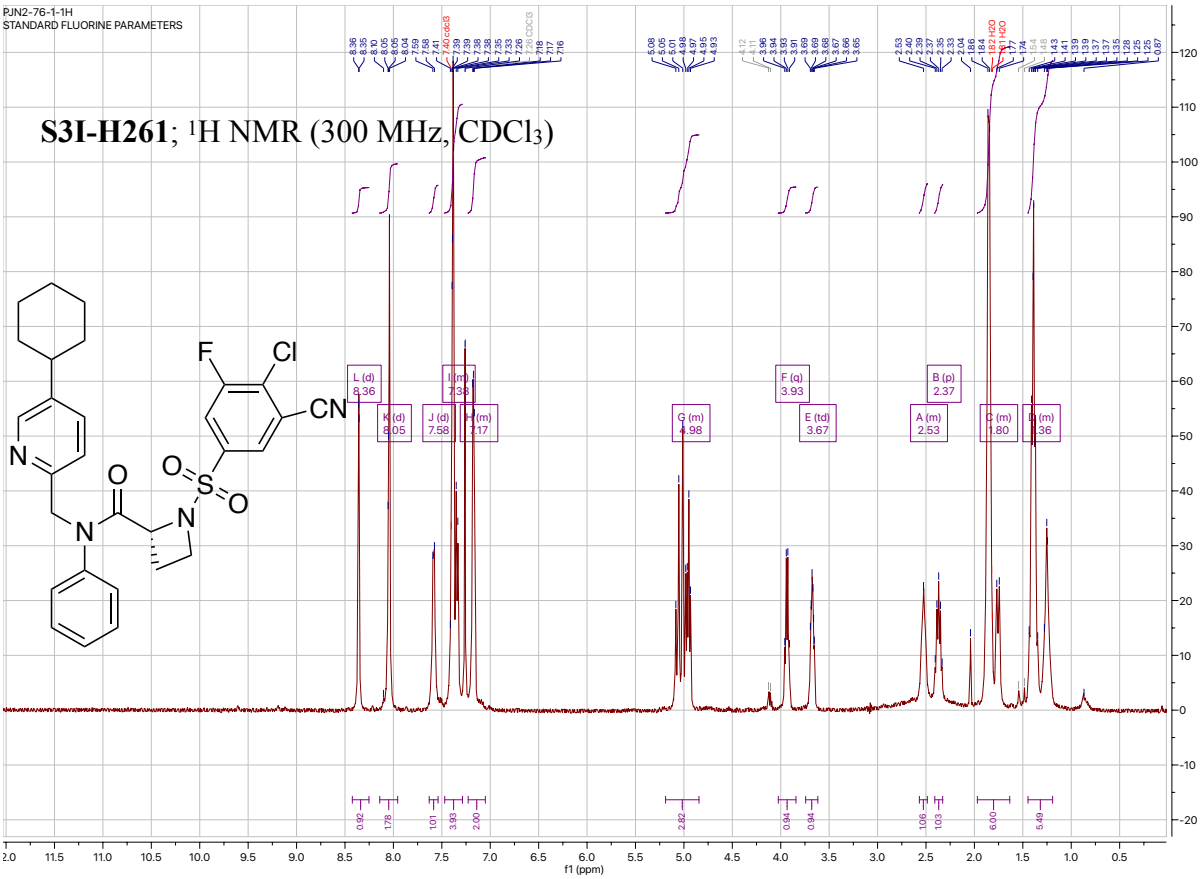


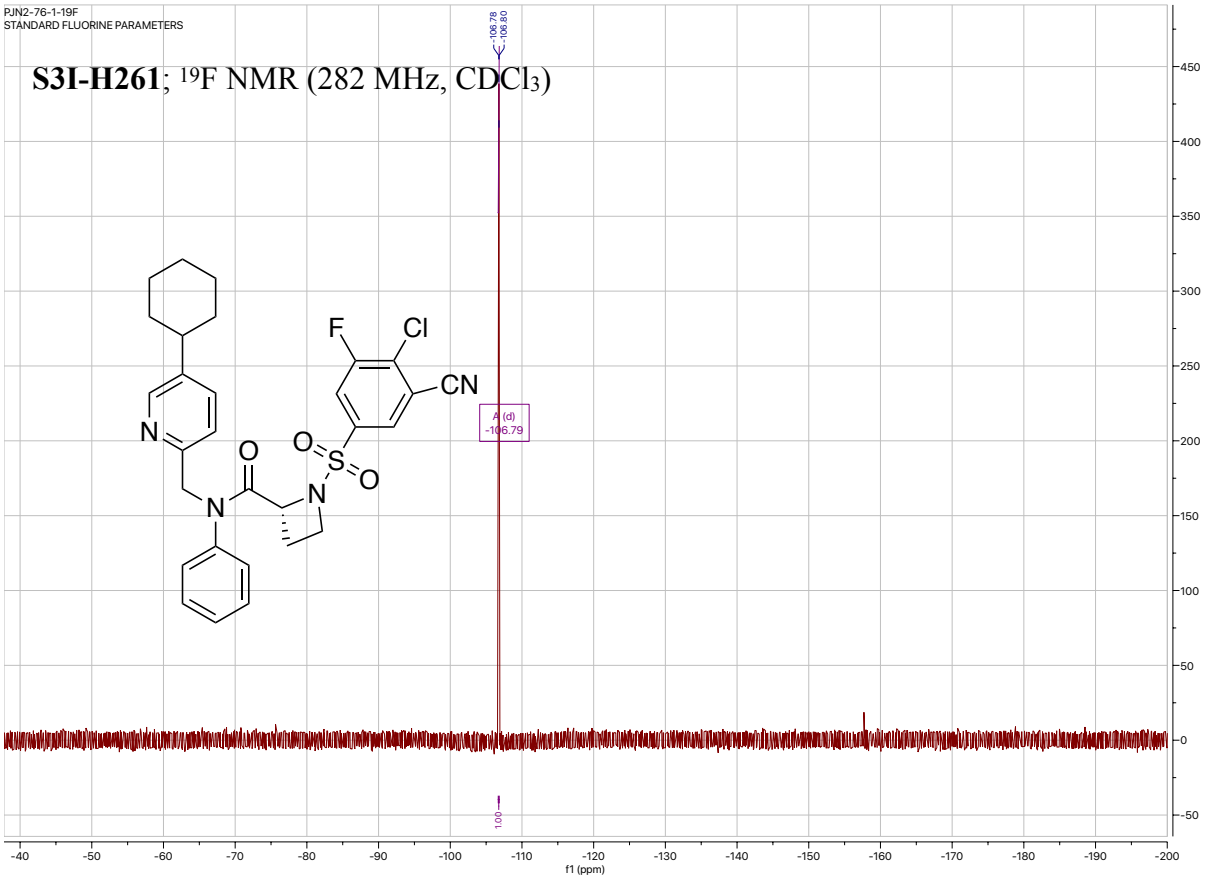
S3I-H247; ^{1}H NMR (300 MHz, $CDCl_3$)

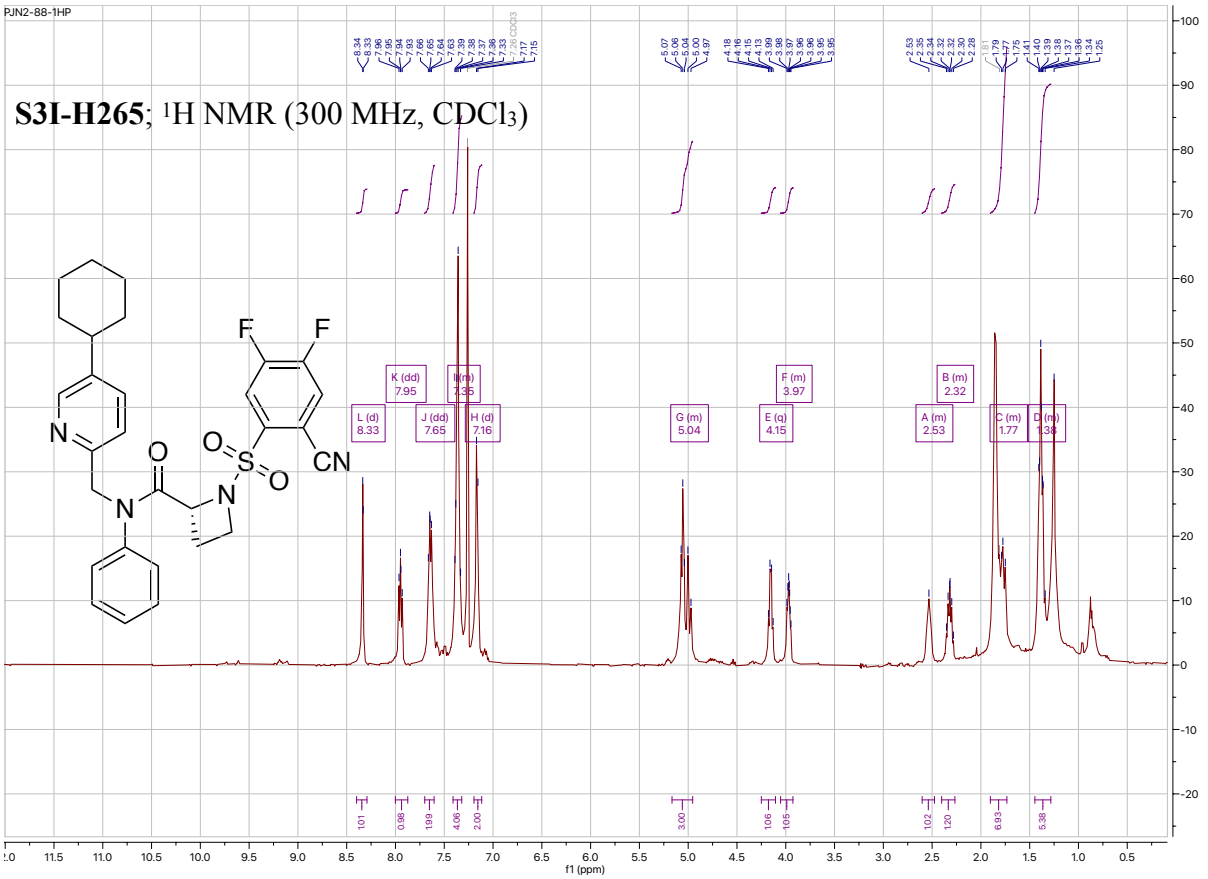

PJN2-7-1-19F
STANDARD FLUORINE PARAMETERS
S3I-H247; 19F NMR (282 MHz, CDCl3)
A (dd) -111.44
B (dq) -135.93
C (tt) -146.85
D (tt) -159.56
f1 (ppm)

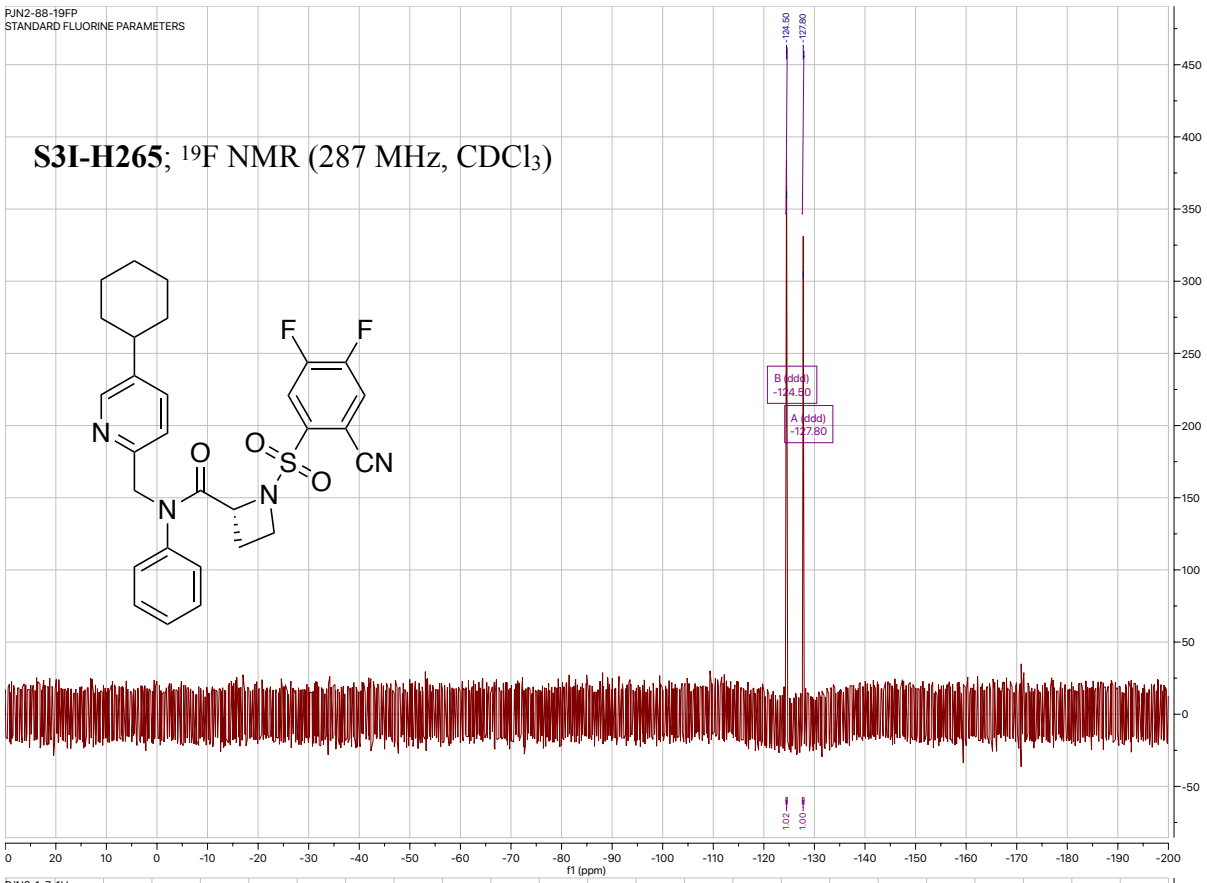

PJN2-46-1-1H
STANDARD FLUORINE PARAMETERS
S3I-H254; 1H NMR (300 MHz, CDCl3)
K (d) 8.37
J (dd) 8.18
N (dd) 8.33
M (d) 7.32
I (dd) 7.56
H (dd) 7.18
L (dd) 7.40
G (m) 6.98
B (ddd) 3.69
A (q) 3.96
D (m) 2.38
C (m) 2.53
E (m) 1.86
F (m) 1.36
f1 (ppm)

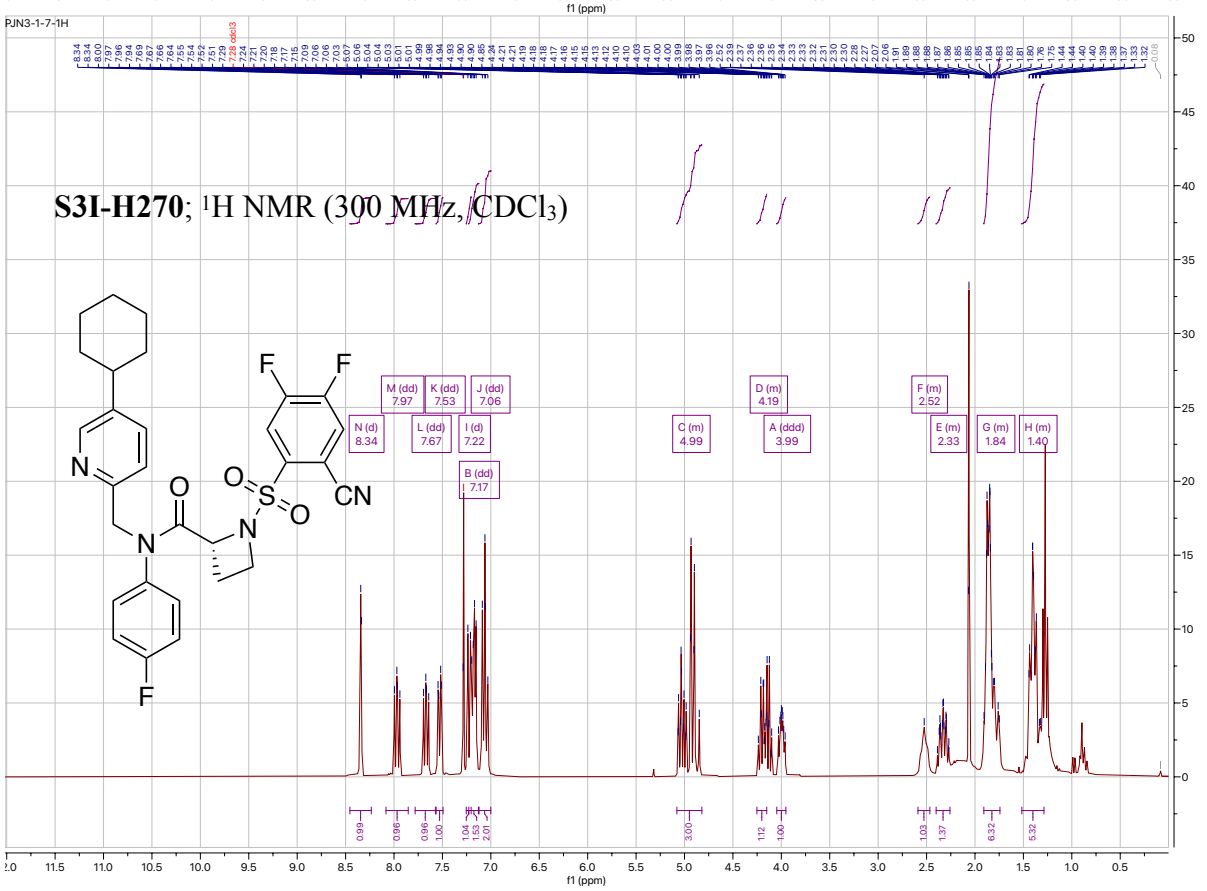

PJN2-46-1-19F
STANDARD FLUORINE PARAMETERS
S3I-H254; 19F NMR (282 MHz, CDCl3)
A (dd)
-101.02
f1 (ppm)

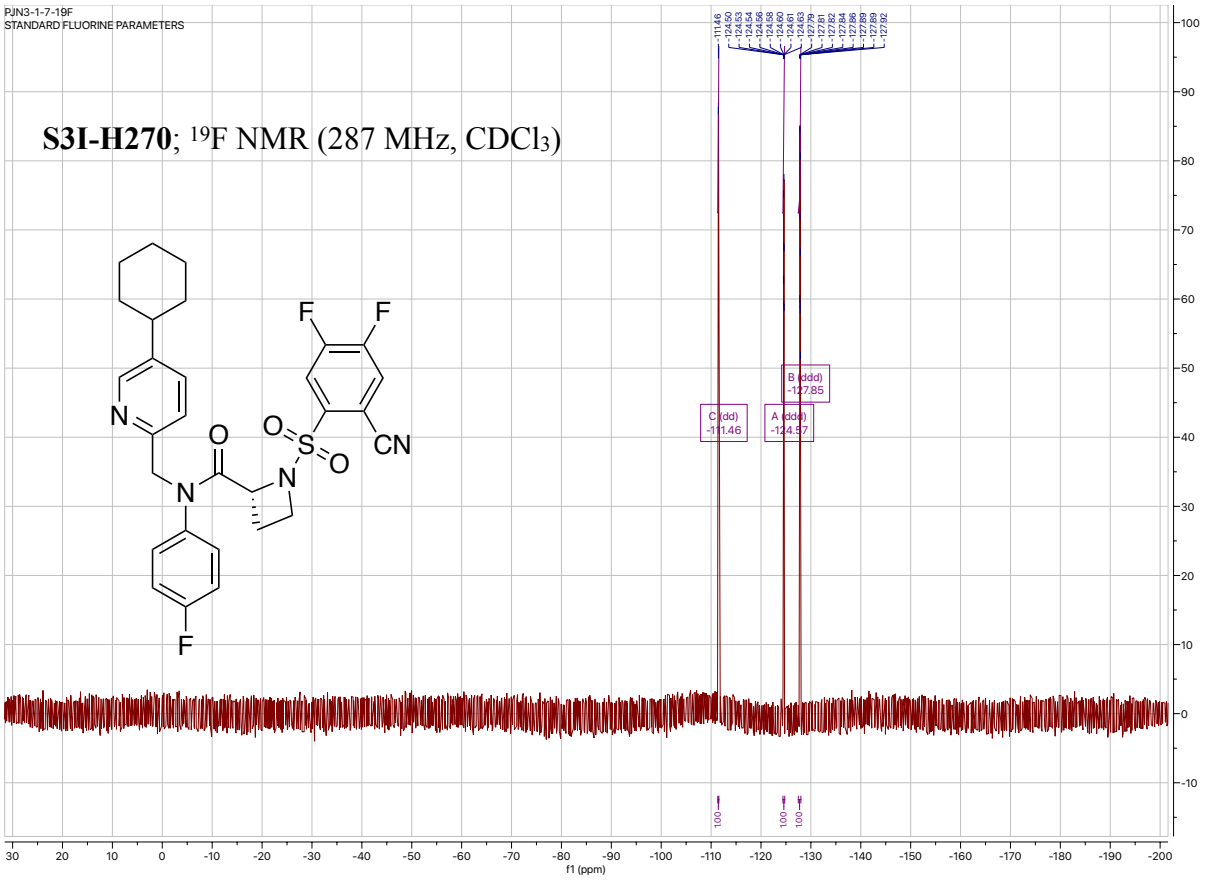

PJN2-76-1-1H
STANDARD FLUORINE PARAMETERS
S3I-H261; 1H NMR (300 MHz, CDCl3)
L (d)
8.36
K (d)
8.05
J (d)
7.58
I (m)
7.39
H (m)
7.17
F (q)
3.93
E (td)
3.67
B (p)
2.37
A (m)
2.53
C (m)
1.80
D (m)
1.36
G (m)
4.98
f1 (ppm)

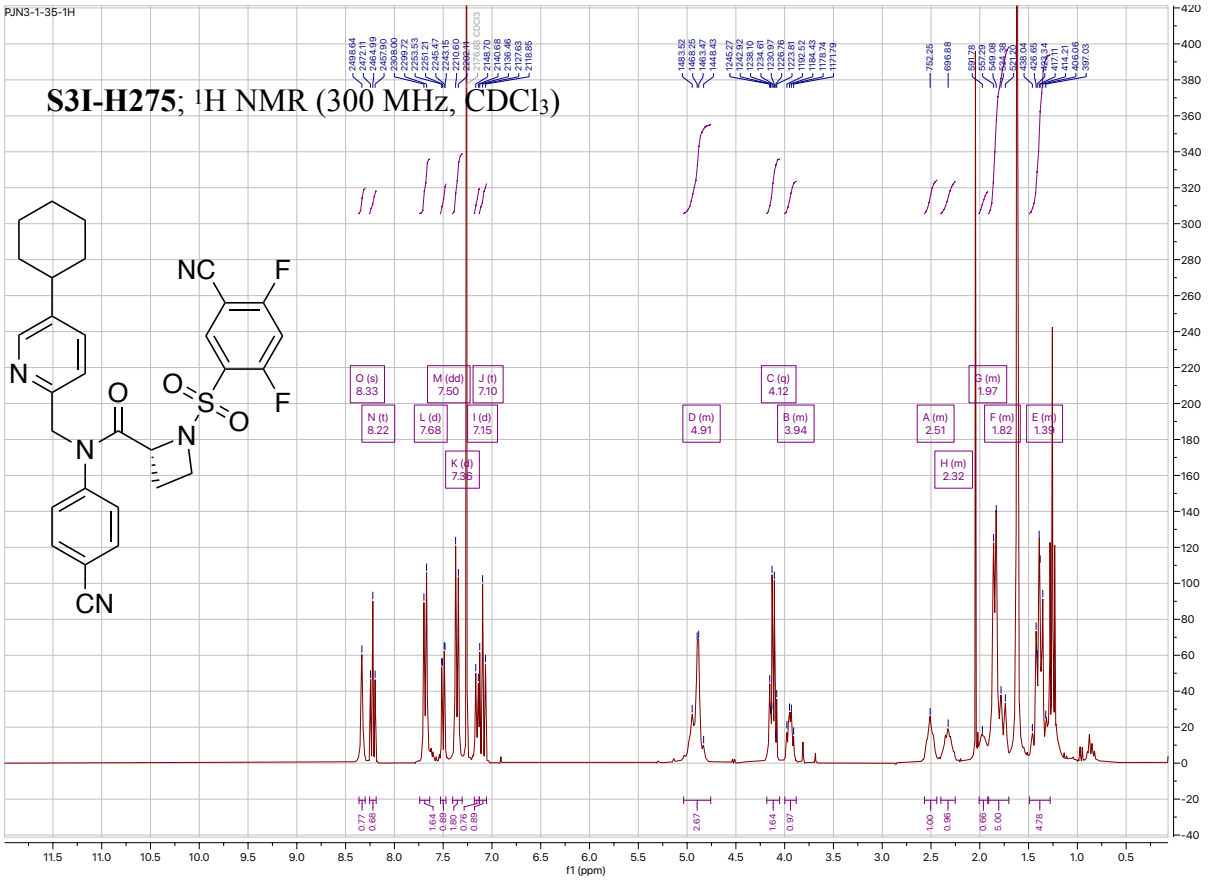

PJN2-76-1-19F
STANDARD FLUORINE PARAMETERS
S3I-H261; 19F NMR (282 MHz, CDCl3)
-106.78
-106.80
A (d)
-106.79
F
Cl
CN
N
O
S
f1 (ppm)

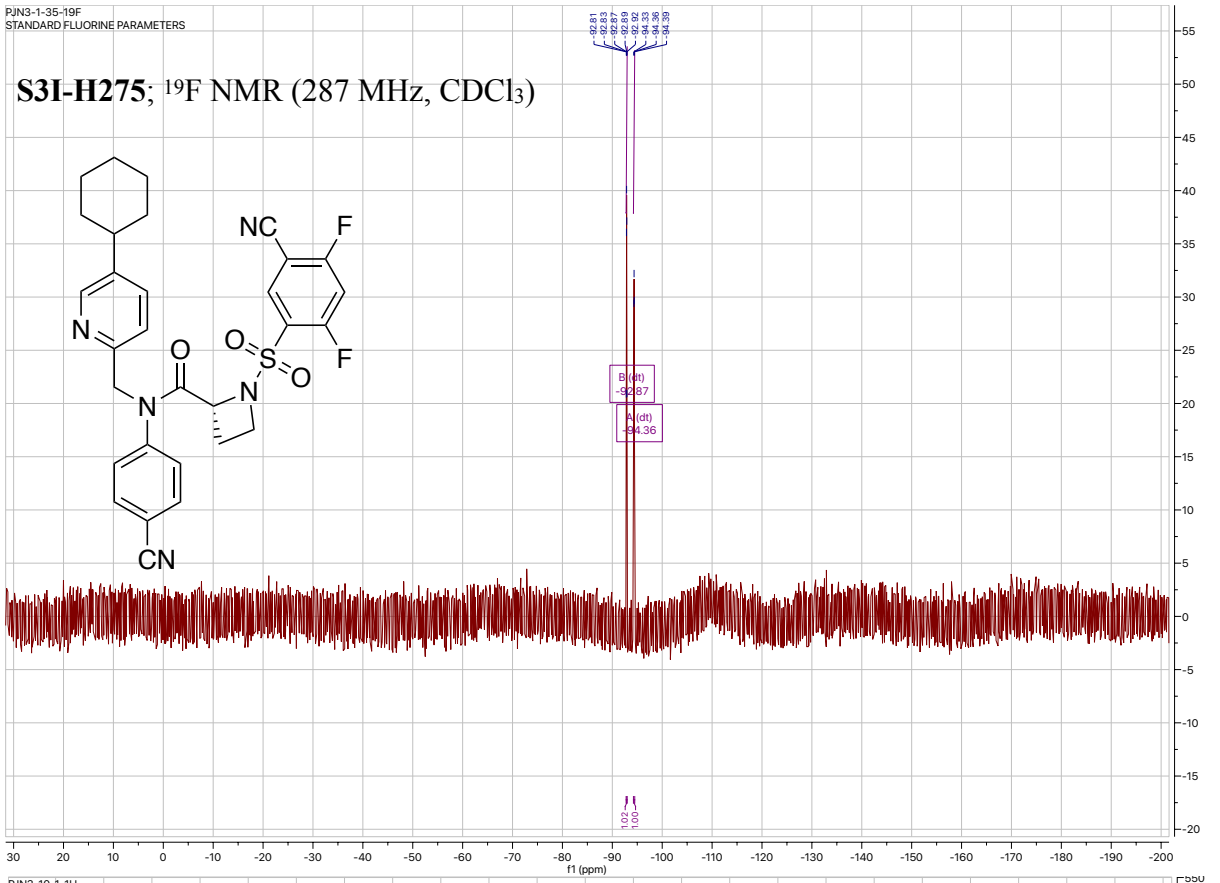

PJN2-88-1HP
S3I-H265; 1H NMR (300 MHz, CDCl3)
L (d)
8.33
K (dd)
7.95
J (dd)
7.65
H (d)
7.16
G (m)
5.04
E (q)
4.15
F (m)
3.97
A (m)
2.53
B (m)
2.32
C (m)
1.77
D (m)
1.34
F
F
CN
N
O
S
f1 (ppm)

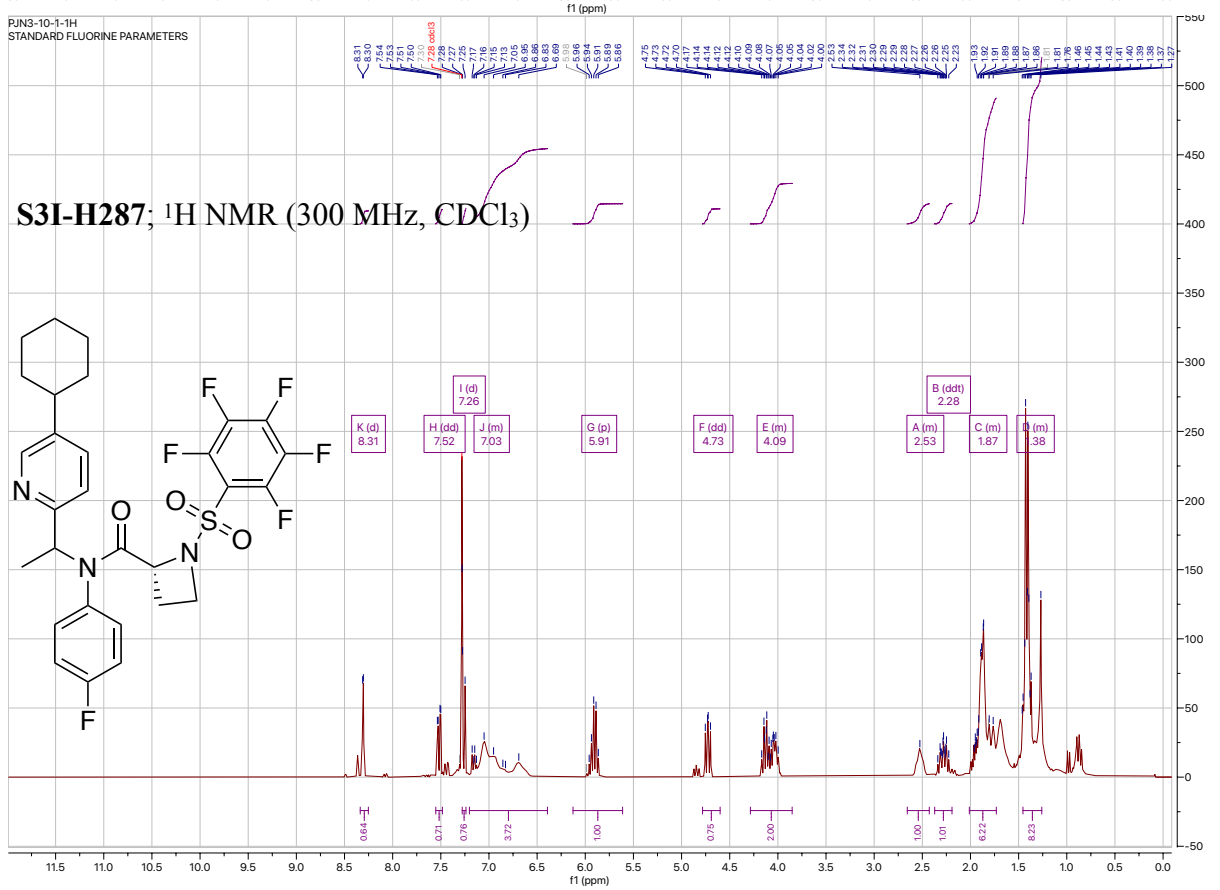

PJN2-88-19FP
STANDARD FLUORINE PARAMETERS
S3I-H265; 19F NMR (287 MHz, CDCl3)
F
F
N
O
O
S
O
N
CN
N
B (ddd)
-124.50
A (ddd)
-127.80
f1 (ppm)

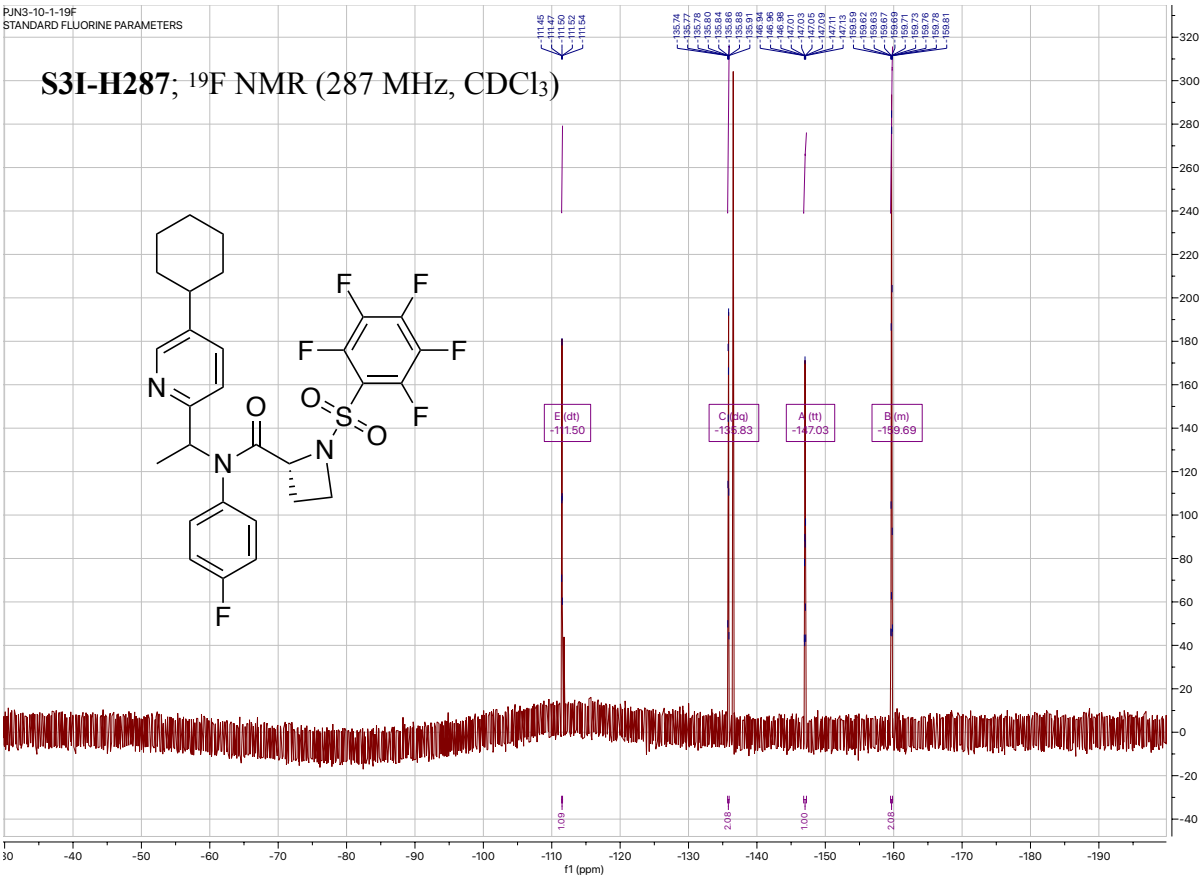

PJN3-1-7-1H
S3I-H270; 1H NMR (300 MHz, CDCl3)
F
F
N
O
O
S
O
N
CN
N
F
M (dd) 7.97
K (dd) 7.53
J (dd) 7.06
N (d) 8.34
L (dd) 7.67
I (d) 7.22
B (dd) 7.17
C (m) 4.99
D (m) 4.19
A (ddd) 3.99
F (m) 2.52
E (m) 2.33
G (m) 1.84
H (m) 1.40
f1 (ppm)

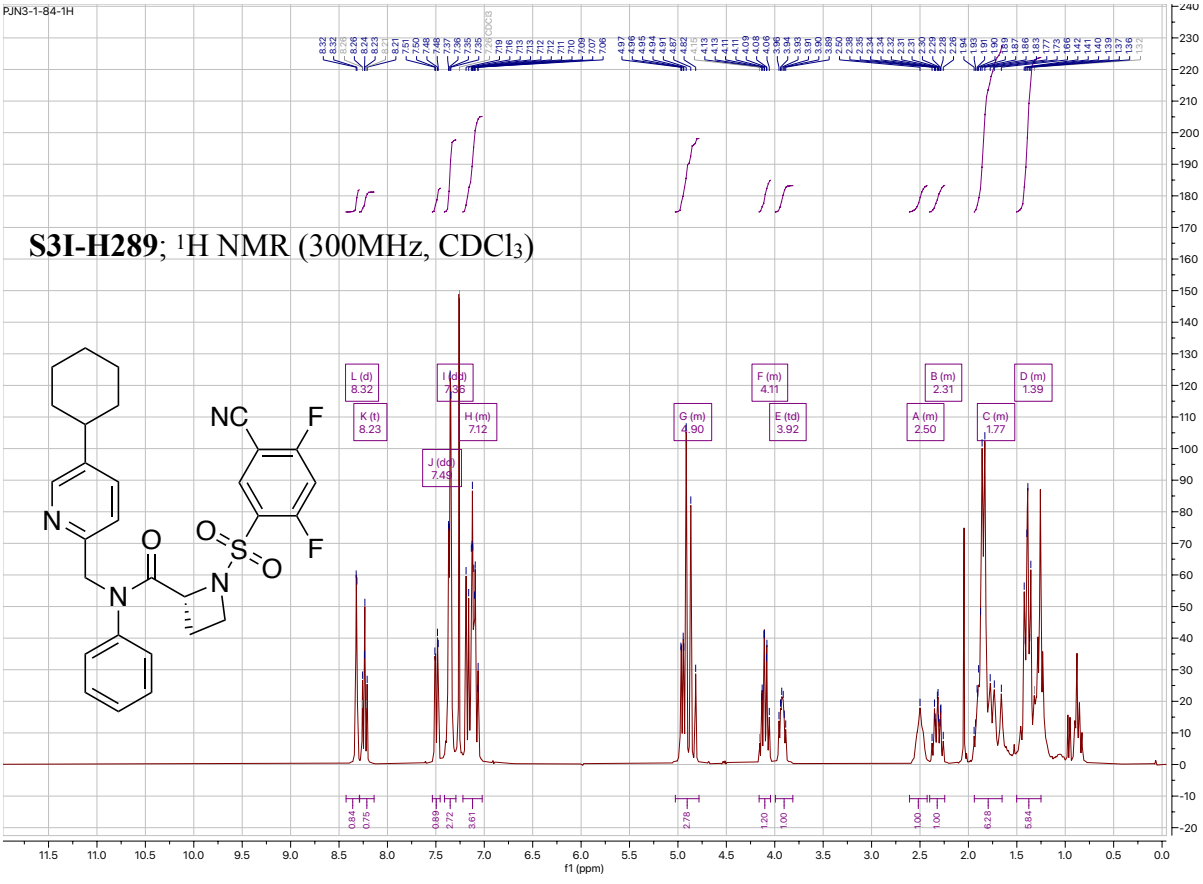

PJN3-1-7-19F
STANDARD FLUORINE PARAMETERS
S3I-H270; 19F NMR (287 MHz, CDCl3)
C (dd)
-111.46
A (ddd)
-124.57
B (ddd)
-127.85
f1 (ppm)

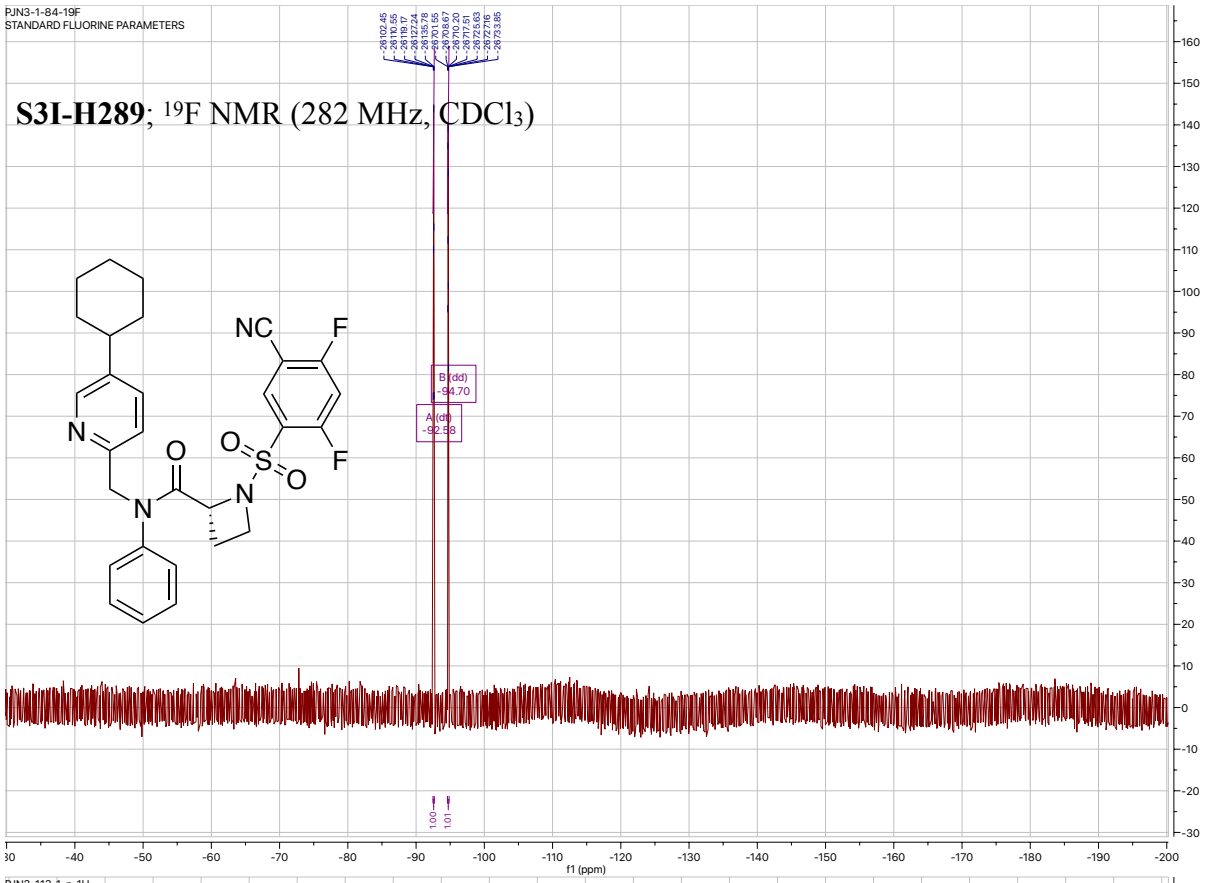

PJN3-1-35-1H
S3I-H275; 1H NMR (300 MHz, CDCl3)
O (s)
8.33
N (t)
8.22
M (dd)
7.50
L (d)
7.68
J (t)
7.10
I (d)
7.15
K (d)
7.36
D (m)
4.91
C (q)
4.12
B (m)
3.94
G (m)
1.97
A (m)
2.51
F (m)
1.82
E (m)
1.39
H (m)
2.32
f1 (ppm)

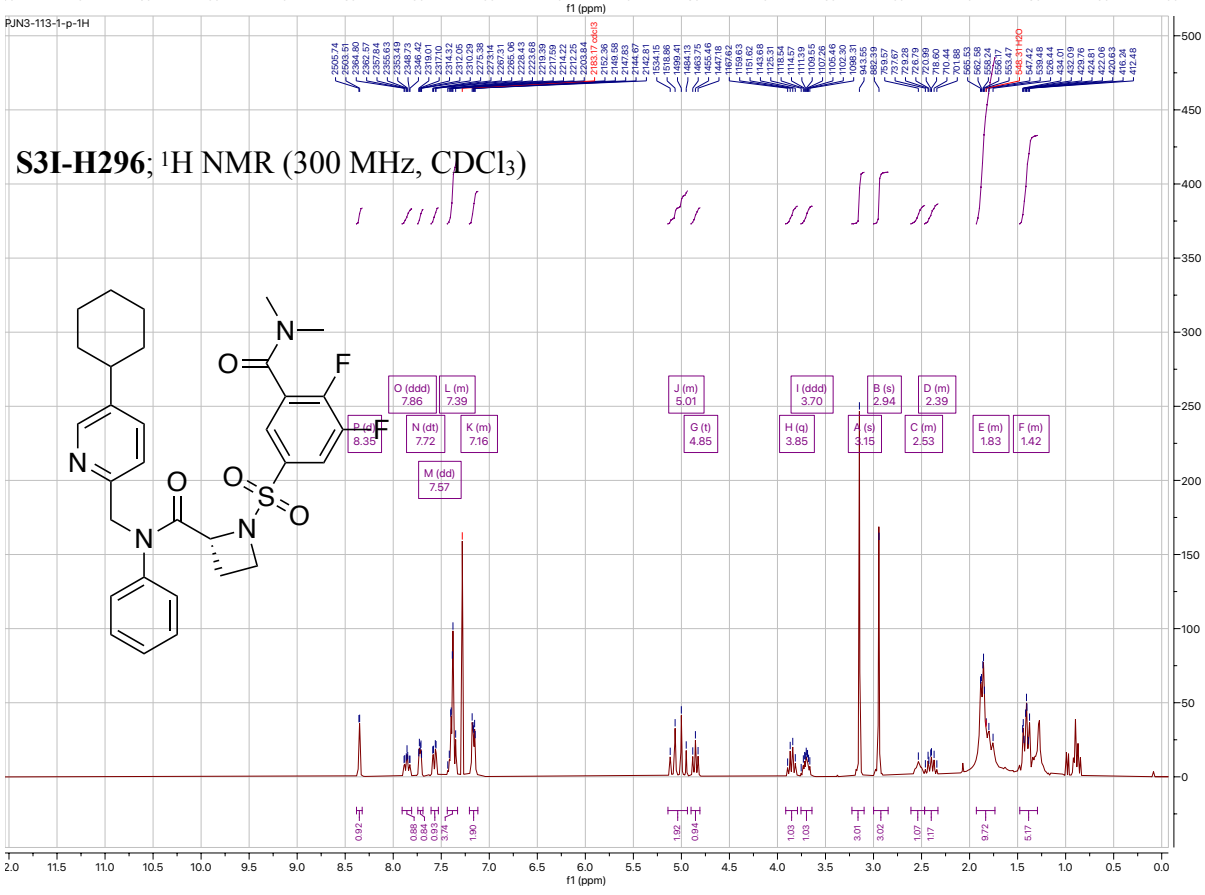

PJN3-1-35-19F
STANDARD FLUORINE PARAMETERS
S3I-H275; 19F NMR (287 MHz, CDCl3)
NC
F
F
O
O
S
O
N
N
N
O
CN
B (dt)
-92.87
A (dt)
-94.36
f1 (ppm)

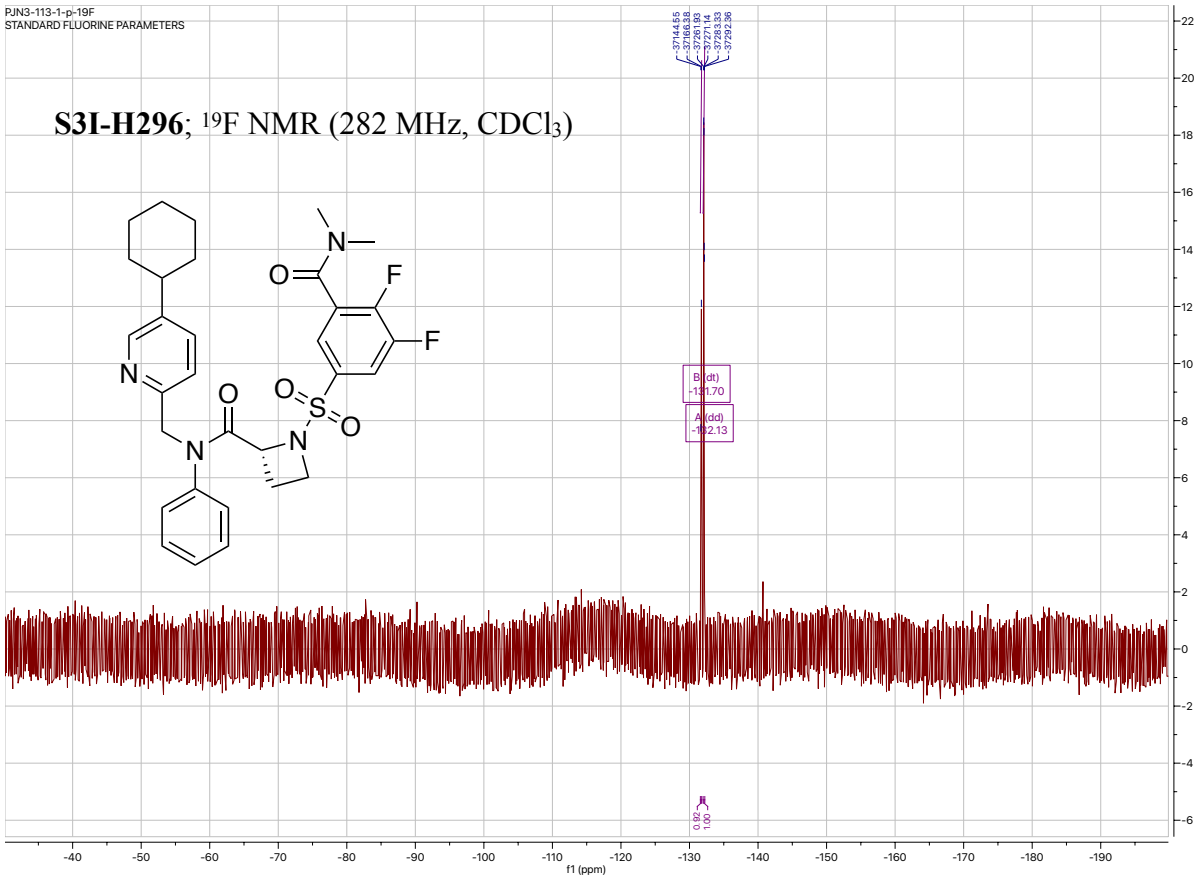

PJN3-10-1-1H
STANDARD FLUORINE PARAMETERS
S3I-H287; 1H NMR (300 MHz, CDCl3)
F
F
F
F
F
O
O
S
O
N
N
N
F
K (d) 8.31
H (dd) 7.52
I (d) 7.26
J (m) 7.03
G (p) 5.91
F (dd) 4.73
E (m) 4.09
A (m) 2.53
C (m) 1.87
B (ddt) 2.28
D (m) 1.38
f1 (ppm)

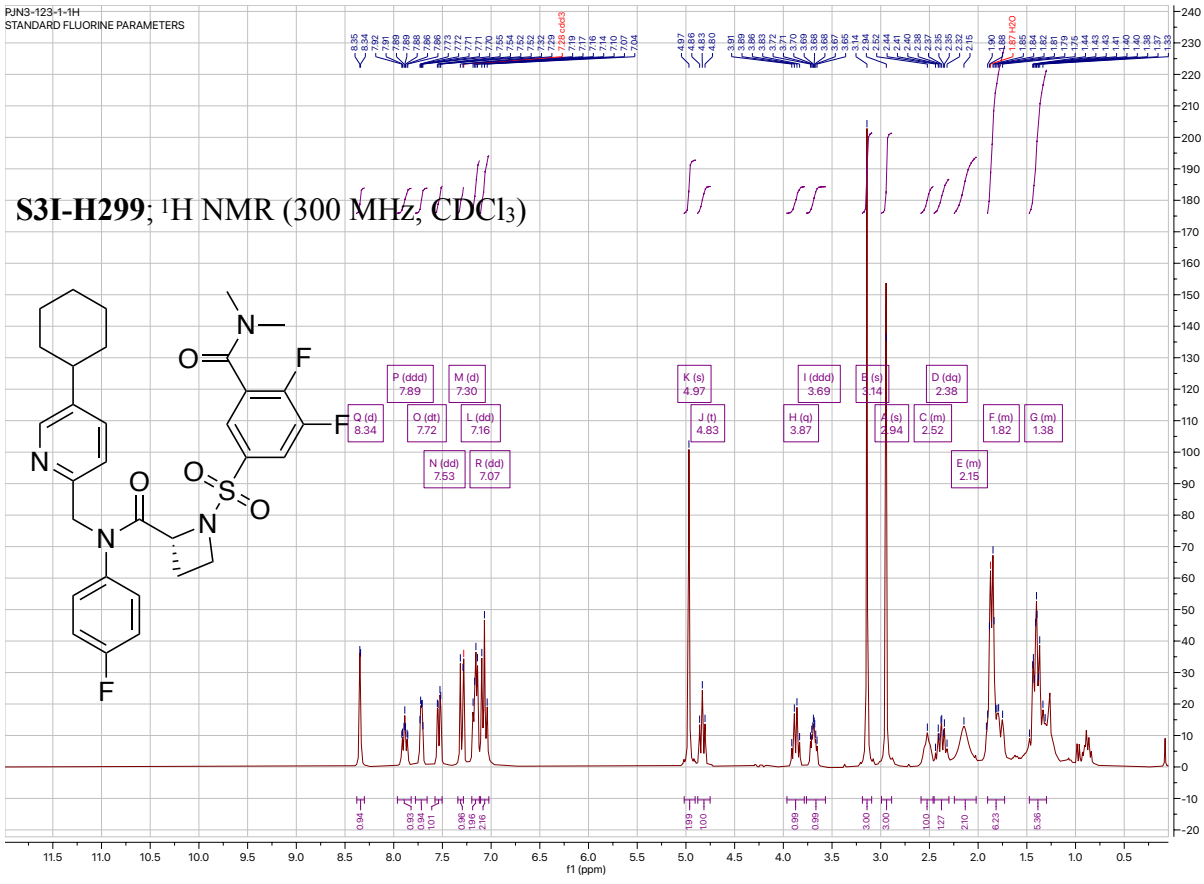

PJN3-10-1-19F
STANDARD FLUORINE PARAMETERS
S3I-H287; 19F NMR (287 MHz, CDCl3)
E (dt) -111.50
C (dq) -135.83
A (tt) -147.03
B (m) -159.69
f1 (ppm)

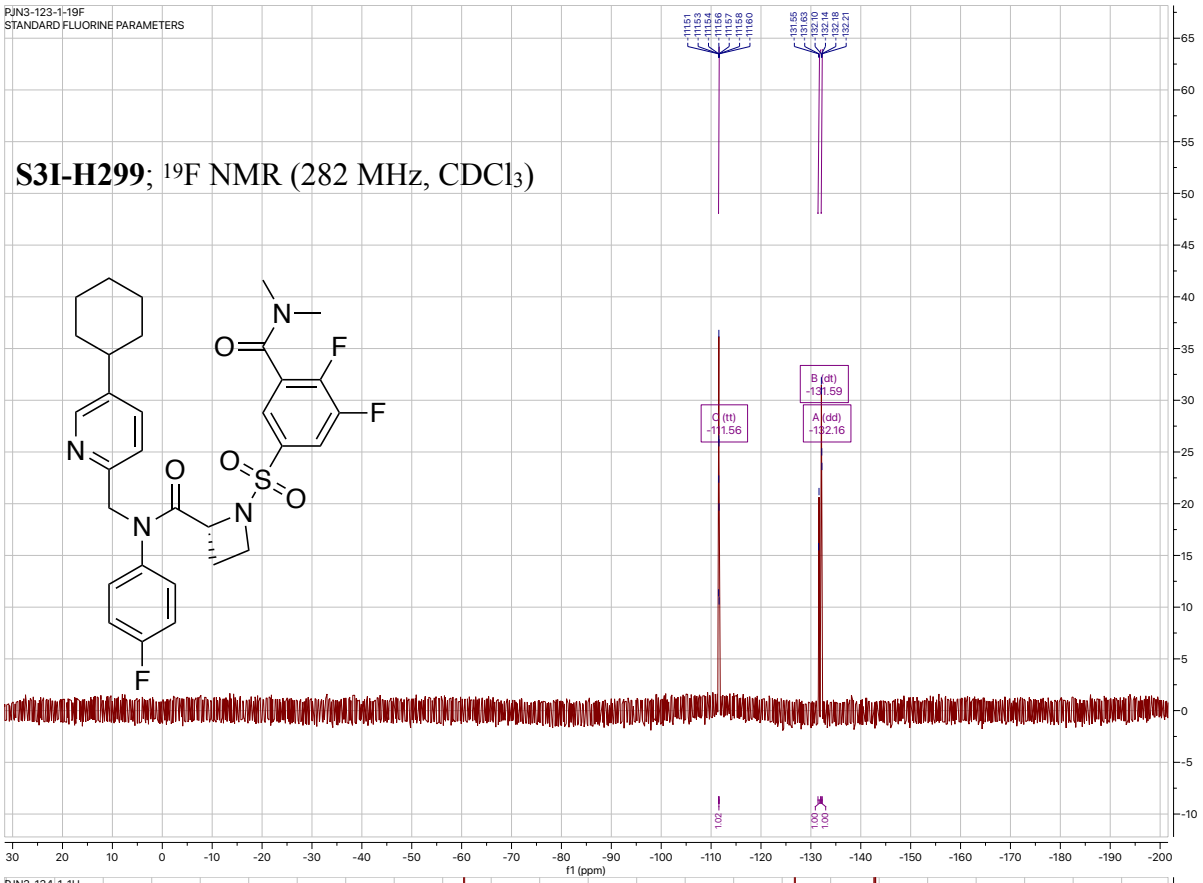

PJN3-1-84-1H
S3I-H289; 1H NMR (300MHz, CDCl3)
L (d) 8.32
K (t) 8.23
I (dd) 7.36
H (m) 7.12
J (dd) 7.49
G (m) 4.90
F (m) 4.11
E (td) 3.92
B (m) 2.31
A (m) 2.50
C (m) 1.77
D (m) 1.39
f1 (ppm)

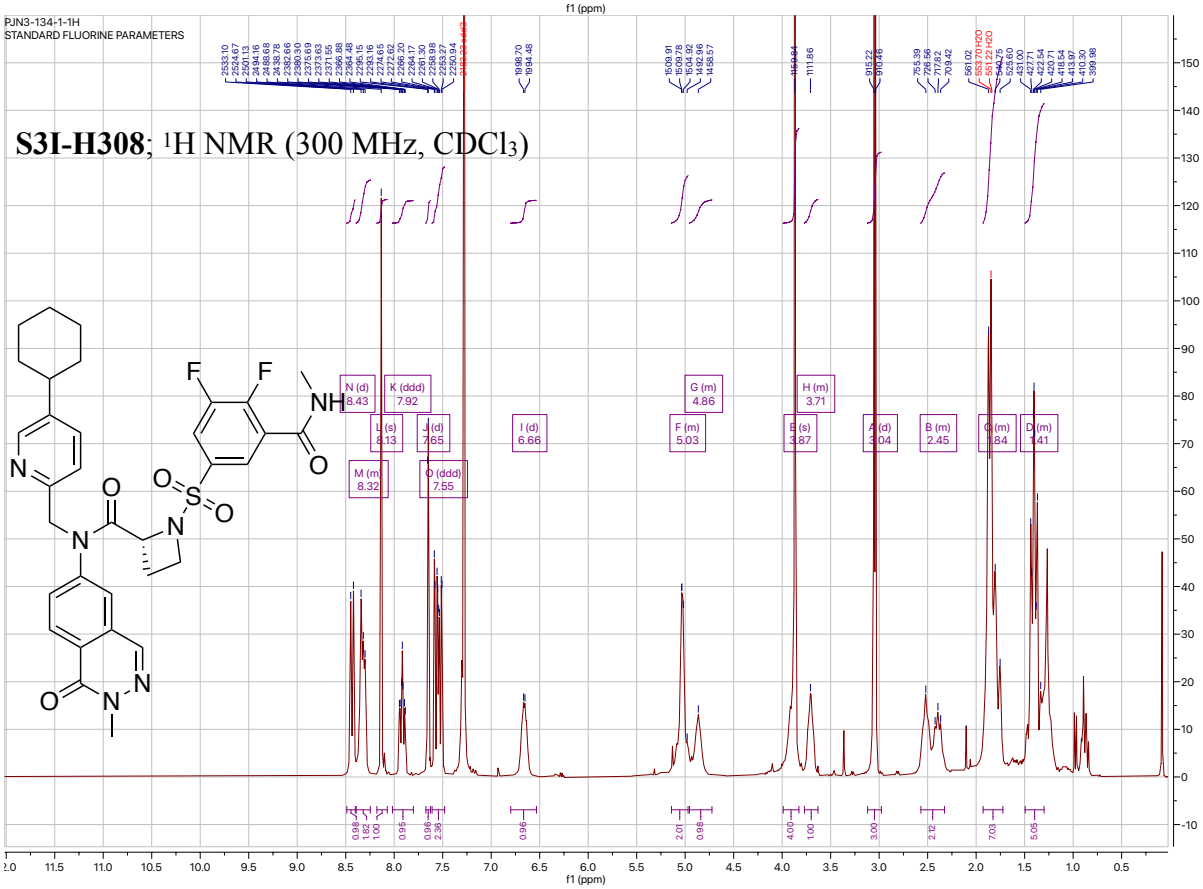

PJN3-1-84-19F
STANDARD FLUORINE PARAMETERS
S3I-H289; 19F NMR (282 MHz, CDCl3)
NC
F
F
O
O
S
O
N
N
N
B (dd)
-94.70
A (dd)
-92.58
f1 (ppm)

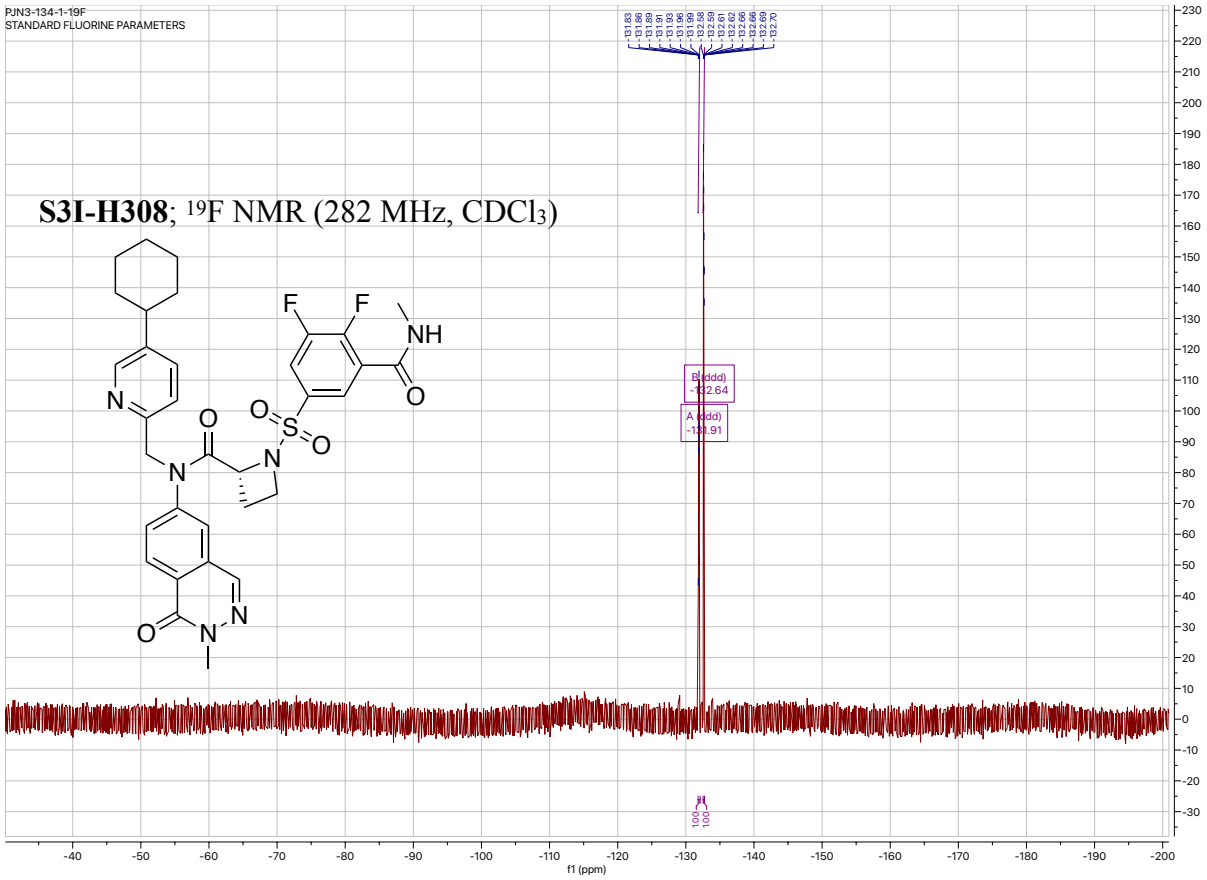

PJN3-113-1-p-1H
S3I-H296; 1H NMR (300 MHz, CDCl3)
N
O
F
F
O
S
O
N
N
N
O (ddd) 7.86
L (m) 7.39
P (d) 8.35
N (dt) 7.72
K (m) 7.16
M (dd) 7.57
J (m) 5.01
G (t) 4.85
I (ddd) 3.70
H (q) 3.85
B (s) 2.94
A (s) 3.15
D (m) 2.39
C (m) 2.53
E (m) 1.83
F (m) 1.42
f1 (ppm)


PJN3-113-1-p-19F
STANDARD FLUORINE PARAMETERS
S3I-H296; 19F NMR (282 MHz, CDCl3)
B (dt)
-131.70
A (dd)
-132.13
f1 (ppm)


PJN3-123-1-1H
STANDARD FLUORINE PARAMETERS
S3I-H299; 1H NMR (300 MHz, CDCl3)
Q (d) 8.34
P (ddd) 7.89
M (d) 7.30
O (dt) 7.72
L (dd) 7.16
N (dd) 7.53
R (dd) 7.07
K (s) 4.97
J (t) 4.83
I (ddd) 3.69
H (q) 3.87
B (s) 3.14
A (s) 2.94
D (dq) 2.38
C (m) 2.52
F (m) 1.82
G (m) 1.38
E (m) 2.15
f1 (ppm)


PJN3-123-1-19F
STANDARD FLUORINE PARAMETERS
S3I-H299; 19F NMR (282 MHz, CDCl3)
C (tt)
-111.56
B (dt)
-131.59
A (dd)
-132.16
f1 (ppm)


PJN3-134-1-1H
STANDARD FLUORINE PARAMETERS
S3I-H308; 1H NMR (300 MHz, CDCl3)
N (d)
8.43
K (ddd)
7.92
L (s)
8.13
J (d)
7.65
I (d)
6.66
M (m)
8.32
O (ddd)
7.55
G (m)
4.86
F (m)
5.03
H (m)
3.71
E (s)
3.87
A (d)
3.04
B (m)
2.45
C (m)
1.84
D (m)
1.41
f1 (ppm)


PJN3-134-1-19F
STANDARD FLUORINE PARAMETERS
S3I-H308; 19F NMR (282 MHz, CDCl3)
F
F
NH
O
N
O
S
O
N
O
N
O
N
N
B ddd)
-132.64
A ddd)
-131.91
f1 (ppm)

Appendix II

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Patient with Advanced Melanoma and Crohn's Disease: a Case Report. J. Hematol. Oncol. **2016**, *9*, 81-84.

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