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Matthew Birnbaum	April 11, 2013

Synthesis and Characterization of *N*-alkyl-3-(2,4-dichlorophenyl)-*N*-fluoroalkyl-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-amines as Potential CRF_1 Receptor PET Radiotracers

By

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Dr. Jeremy J. Weaver, PhD
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An abstract of
A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
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Department of Chemistry

2013

Abstract

Synthesis and Characterization of *N*-alkyl-3-(2,4-dichlorophenyl)-*N*-fluoroalkyl-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-amines as Potential CRF_1 Receptor PET Radiotracers

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N-alkyl-3-(2,4-dichlorophenyl)-*N*-fluoroalkyl-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-amines were synthesized from commercial starting materials. As hypothesized, binding assays indicate that this class of molecules is a strong inhibitor of the CRF_1 receptor (K_i= 0.72-33 nM). As lipophilicity of the 7-amine alkyl chains increases, binding strength increases. One molecule has been successfully radiolabeled indicating that these molecules are potential CRF_1 receptor PET radiotracers.

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At the outset of college, I had no intention of becoming a chemistry major, but when I was introduced to organic chemistry, my eyes were opened to a new way of perceiving the world. My research experiences have given me the opportunity to travel the country and make many new friends. If it were not for Emory University and its chemistry department's dedication to undergraduate research, I would not be the person I am receiving a diploma.

I would like to thank Dr. Simon B. Blakey for giving me my introduction to organic chemistry as my first professor and principle investigator. His early guidance and team of graduate students laid the foundation for my synthetic skills.

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I would like to thank Dr. Stefan A. Lutz and Dr. Jeremy J. Weaver for agreeing to be on my honors thesis committee. They are two of the chemistry department's strongest professors whose lessons and guidance continuously inspired me to question and discover.

Lastly, I would like to thank Dr. Jeffrey S. Stehouwer for designing and advising this thesis project. He has been an unnaturally patient and wise mentor. I have become infused with his excitement for radio-medicinal chemistry.

Table of Contents

Equations, Figures, Tables, and Schemes

1. Introduction

1.1 Positron Emission Tomography

Positron emission tomography (PET) utilizes radioactivity to monitor protein-molecule interactions *in vivo*. A molecule with a known binding profile is labeled with ^{11}C, ^{13}N,^{15}O, ^{18}F, ^{76}Br, or ^{124}I and injected into a body. As the radioactive isotope decays, a proton decays to a neutron with the emission of a neutrino and a positron, the antiparticle of an electron (eq. 1). When a positron collides with an electron, an annihilation event occurs, which sends two 511 keV γ-photons in 180° opposing directions (eq. 2). The energy of each photon is derived from Einstein's famous energy equation (eq. 3). Detectors on a PET scanner are simultaneously struck by the two photons (Figure 1). With enough "lines," a two dimensional picture can be drawn of the concentration of the labeled molecule throughout the body. If the tracer binds selectively to a protein, the location and density of γ radiation can give a quantitative measure of protein concentration.[1]

$$ {}^{1}_{1}\mathrm{p} \rightarrow {}^{1}_{0}\mathrm{n} + {}^{0}_{1}\mathrm{e} + \nu_e \tag{1} $$

$$ {}^{0}_{1}\mathrm{e} + {}^{0}_{-1}\mathrm{e} \rightarrow 2{}^{0}_{0}\gamma \tag{2} $$

$$ \mathrm{E} = \mathrm{mc}^2 = \frac{(9.109 \times 10^{-31}\mathrm{kg})(2.9988 \times 10^{8}\mathrm{m/s})^2}{1.602 \times 10^{-16}\mathrm{J/keV}} = 511\ \mathrm{keV} \tag{3} $$

PET has become a leading tool in monitoring central nervous system (CNS) activity because it is noninvasive with high image resolution.[2] A viable CNS radiotracer should cross the blood brain barrier (BBB), bind selectively to the protein of interest, and survive metabolism for the duration of a PET scan. Tracers that adhere to these

requirements can then be used to study biodistribution, target validation, and changes in biomarkers.[1]

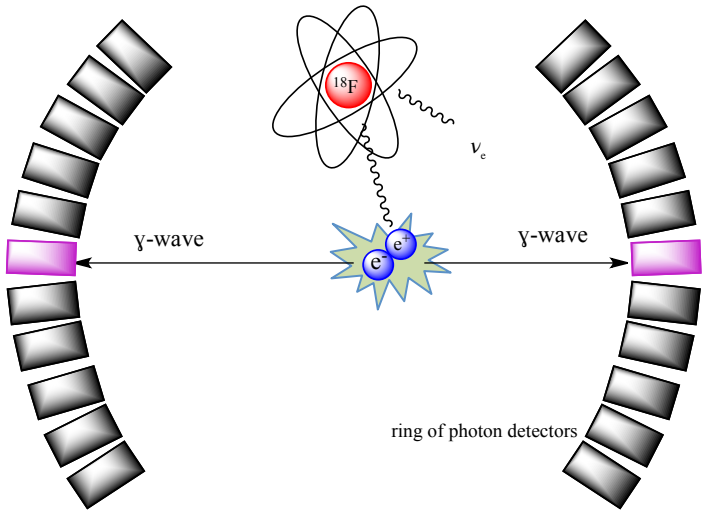


Figure 1. Simplistic PET Scan model

^{18}F is made in a cyclotron by bombarding [^{18}O]water with protons to give H^{18}F (eq. 4). The radioactive hydrofluoric acid is then neutralized with K_2CO_3 to give K^{18}F which is employed for radiolabeling.[3] Reactions are conducted at high temperatures to expedite the conversion.[4] Only 5-15 mCi of tracer are needed for a PET study, so chemical yields are secondary to making the tracer.

$$^{18}_{8}O + {}^{1}_{1}p \rightarrow {}^{18}_{9}F + {}^{1}_{0}n \qquad ^{18}O(p,n)^{18}F \tag{4}$$

Purification is conducted by HPLC. The retention time of the radiolabeling precursor and the cold product (^{19}F) are assessed prior to labeling. The cold product and radiotracer will have the same retention time in the same solvent system. This data is used to identify and purify the PET tracer.

The scientific community has developed a host of techniques to label with ^{18}F.[4] Aromatic labeling can be done by nucleophilic substitution with a good leaving group *ortho* or *para* to a strong electron-withdrawing group. Aliphatic labeling mirrors an S_N2

reaction in a polar, aprotic solvent. Not all molecules are amenable to a single step labeling, so a labeling agent, in which a simple molecule is labeled with ^{18}F and then attached to the tracer of interest, is commonly used.

1.2 Crossing The Blood Brain Barrier

For a CNS tracer to be effective, it must pass the BBB. The body has many mechanisms to prevent xenobiotic materials from damaging the brain. Narrow capillaries studded with efflux transporters make it difficult for large molecules to diffuse into the brain.[5]

Small (<450 Da), lipophilic molecules are best suited to enter the brain.[5] While the more lipophilic molecules are better at crossing the BBB, a too lipophilic molecule will not be soluble in the blood and be incapable of crossing the BBB. Molecules with a log *P* (a measurement of lipophilicity based on a molecule's preference to octanol over a buffer) between 2.0-3.5 have been shown to be the most effective CNS therapeutics.[6]

1.3 Corticotropin-Releasing Factor

Discovered in 1981 by the Vale group, Corticotropin-releasing factor (CRF) is a hypothalamus-derived peptide responsible for signaling the secretion of adrenocorticotropic hormone (ACTH) in the pituitary gland, which subsequently signals the release of cortisol in the adrenal glands.[7] Behavioral and physiological studies in rats post intracerebroventricular injection of CRF show the hormone's association with stress.[8] Patients suffering with depression, anxiety disorders, anorexia, and Alzheimer's disease have had positive correlation with heightened levels of ACTH.[7] The scientific

community aims to regulate the CRF signaling pathway for therapeutic purposes with CRF receptor antagonists.[7]

The CRF receptors (CRF_1, $CRF_{2\alpha}$, $CRF_{2\beta}$, $CRF_{2\gamma}$) are G protein coupled receptors found throughout the CNS and peripheral organ systems.[7] CRF_1, the most abundant, is found primarily in the neocortex and cerebellum while the CRF_2 class of receptors is distributed throughout the body. Urocortin in humans, sauvagine in frogs, and urotensin I in sucker fish are other known peptidic antagonists of the receptors.[9] Some cancer lines have shown heightened expression of CRF receptors, indicating a potential therapeutic or diagnostic target.[10]

1.4 Non-peptidic CRF_1 Receptor Antagonists

For a CRF antagonist to be an effective CNS therapy, the molecule must bind strongly and selectively to its intended receptor. While a variety of peptidic antagonists have been developed for the CRF_1 receptor, these peptides are unfortunately too large to cross the BBB and are therefore poor therapeutics. Instead, the scientific community has worked to develop a broad range of small molecule antagonists for CRF.[9]

Researchers at Sanofi, DuPont, Pfizer, Bristol-Myers Squibb, Neurocrine, and many other pharmaceutical companies have conducted numerous structure activity relationship (SAR) studies to elucidate the ideal moieties for a CRF_1 antagonist (Figure 2).[9] A few themes remain constant. The core of the molecule is a heterocycle (typically bicyclic) with an sp^2-hybridized nitrogen. A tri- or di-substituted aryl ring hangs from the core. Lastly, a lipophilic group must be para to the sp^2-hybridized nirogen. Ring size,

number of nitrogens, and substitution around the aryl ring or core can be modified to fine tune the molecule's lipophilicity and binding affinity.

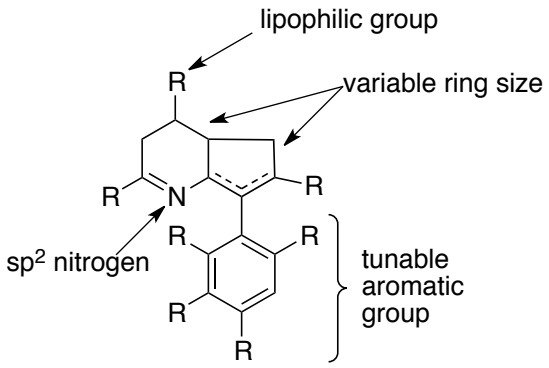
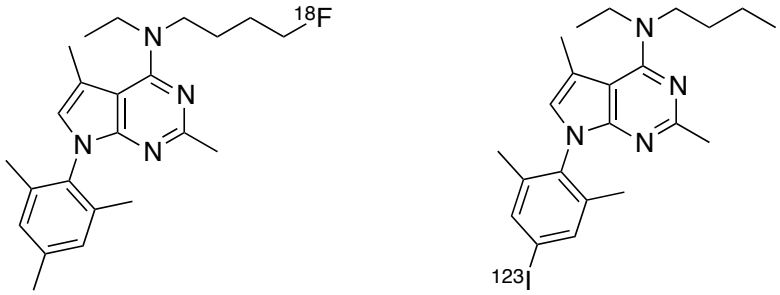


Figure 2. Generalized CRF_1 receptor antagonist

1.5 CRF_1 PET Tracer Developments

The Goodman group radiolabeled the first CRF_1 antagonists in 2000.[11] Derivatives of butyl[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[*2,3-d*]pyrimidin-4-yl]ethylamine, a molecule developed by Pfizer with high affinity for CRF_1 (*Ki*=5.5 nM), were labeled with ^{18}F and ^{123}I (Figure 3). ^{18}F was added in a single step to the terminus of the *N*-alkyl chain by an S_N2 reaction with a methylsulfonate leaving group to afford [^{18}F]FBPPA (**1**). ^{123}I labeling was added by electrophilic iododestannylation on the aromatic ring to afford [^{123}I]IBPPA (**2**).



1 **2**

Figure 3. First radiolabeled CRF_1 PET tracers

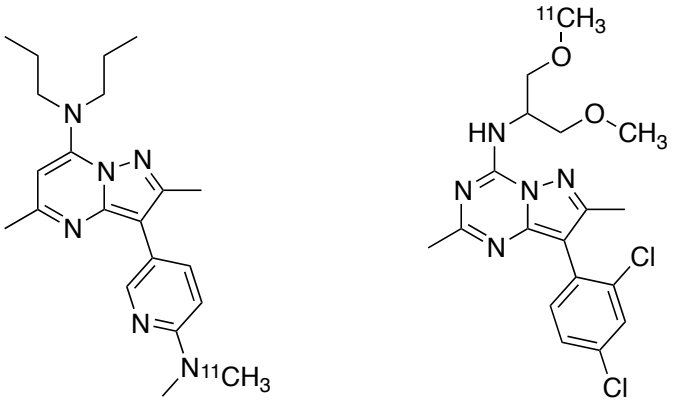


Figure 4. PET tracers synthesized by the Mann group

In vitro assays showed that the cold compounds bound strongly to CRF_1. The radiolabeled compounds were evaluated in biodistribution studies in rats, where it was found that that the compound was not present in the brain (**1**: 0.18% ID/g; **2**: 0.05% ID/g). The group hypothesized that the molecules were too lipophilic to cross the BBB.

The Mann group synthesized [N-methyl-^{11}C]-3-[(6-dimethylamino)pyridine-3-yl]-2,5-dimethyl-*N*,*N*-dipropylpyrazolo[1,5-a]pyrimidine-7-amine (**3**) and [*O*-methyl-^{11}C]-4-(1,3-dimethoxy-2-propylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)[1,5-a]pyrazolo-1,3,5-triazine (**4**) as potential radiotracers.[12,13] Both compounds displayed strong binding affinity (**3** K_i=4.0 nM; **4** K_i=3.2 nM) and a reasonable lipophilicity (**3** log *P*=4.0; **4** log *P*=3.2).[14] PET studies in baboons showed that these molecules were retained in the brain, but had low selectivity for CRF_1. Furthermore, the PET tracers were rapidly metabolized in the body.

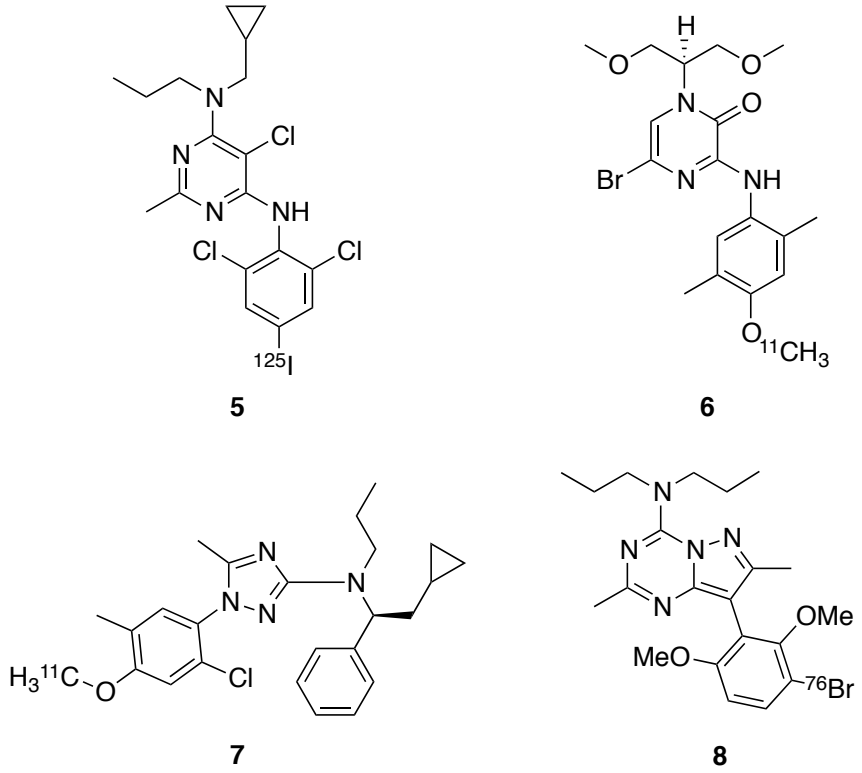


Figure 5. Previously synthesized and biologically assessed radio tracers.

Other groups have continued to develop PET tracers for CRF_1 using ^{18}F, ^{11}C, and ^{76}Br labeling (Figure 5).[15-18] However, none have been successful tracers due to lipophilicity, selectivity, and metabolic issues.

1.6 PET Tracer Design

Our group chose to develop fluorinated derivatives of 3-(2,4-dichlorophenyl)-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-amine as potential PET tracers (Figure 6). Previous binding studies have shown this class of compounds to have reasonably strong affinity to CRF_1 *in vitro* (K_i=3.2 nM).[19] The lipophilicity of this class of molecules has not been studied; however, the atomic make up closely mimics **1** with the exception of the aryl ring. Disubstituted chlorines are hypothesized to be more hydrophilic than trisubstituted

methyl groups. In addition, these molecules' atomic masses range from 321-395 Da, an appropriate mass for crossing the BBB.

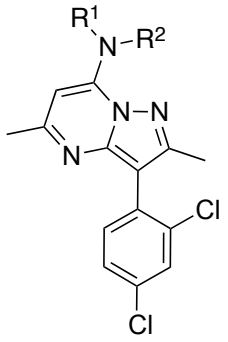


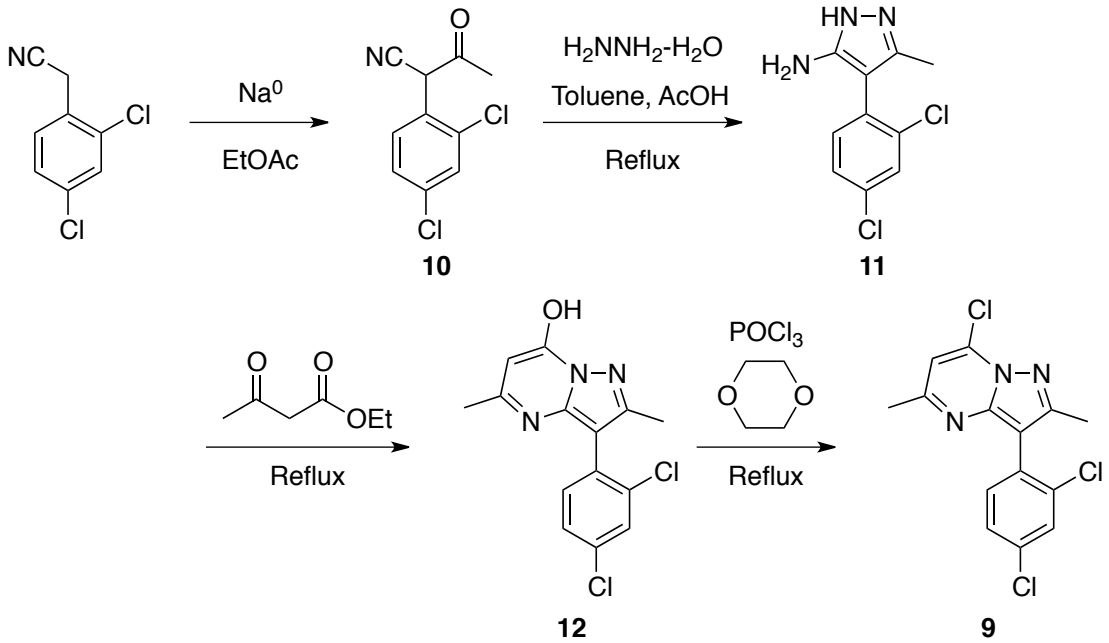
Figure 6. Generalized structure for molecules synthesized

Our synthetic scheme is designed for a divergent synthesis. From the 7-*N* chlorinated compound, a large library of fluoroalkyl amines is possible. SAR studies for the optimal tertiary amine are facilitated by our methodology. The fluorinated chains are added as a final step, a necessary condition for radiolabeling.

2. Synthesis

Synthesis of the 7-chloro-3-(2,4-dichlorophenyl)-2,5-dimethylpyrazolo[1,5-*a*]pyrimidine (**9**) core is illustrated in Scheme 1.[19,20] Briefly, sodium metal extracts a proton from the benzylic carbon of 2-(2,4-dichlorophenyl)acetonitrile. The lone pair reacts with ethyl acetate in a nucleophilic addition-elimination reaction to yield 1-cyano-1-(2,4-dichlorophenyl)propan-2-one (**10**). Treatment of the ketonitrile with hydrazine monohydrate under acidic conditions in refluxing toluene causes cyclization to yield 4-(2,4-dichlorophenyl)-3-methyl-1*H*-pyrazol-5-amine (**11**). The aminopyrazole is then refluxed in ethylacetoacetate (AcAcOEt) to afford 3-(2,4-dichlorophenyl)-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-ol (**12**). Dehydration and chlorination to yield **9** is accomplished using phosphoryl chloride.

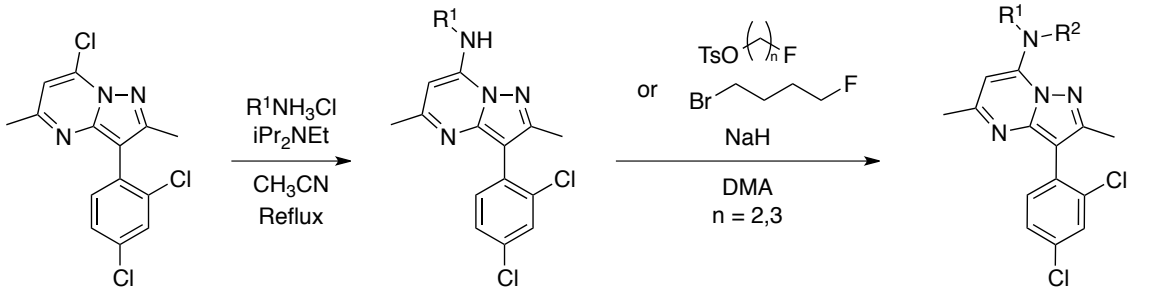
Scheme 1



The 7-position amine was assembled stepwise as illustrated in Scheme 2. In refluxing acetonitrile, **9** reacts with a primary amine salt in the presence of base to make a

3-(2,4-dichlorophenyl)-*N*-alkyl-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-amine (**13 - 16**). Fluoroalkyl tosylate or fluoroalkyl bromide (dependent on commercial availability) is bound to the 7-position amine by an S_N2 reaction to yield a completed *N*,*N*-dialkyl-3-(2,4-dichlorophenyl)-*N*,*N*-diethyl-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-amine (**17 - 22**). A strong base such as sodium hydride is needed to abstract the proton.

Scheme 2



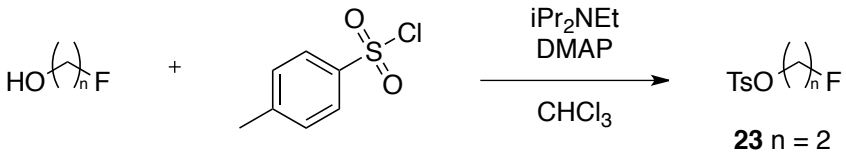
9

13 R^1 = Me
14 R^1 = Et
15 R^1 = Pr
16 R^1 = FEt

17 R^1 = Me R^2 = FPr
18 R^1 = Me R^2 = FBu
19 R^1 = Et R^2 = FEt
20 R^1 = Et R^2 = FPr
21 R^1 = Pr R^2 = FEt
22 R^1 = Pr R^2 = FPr

Fluoroethyl tosylate (**23**) and fluoropropyl tosylate (**24**) were made from their respective primary alcohols. Separate reactions dissolving the fluoroalcohol, tosylchloride, iPr_2NEt, and DMAP in chloroform and stirring for over three days afforded the tosylates (Scheme 3).

Scheme 3

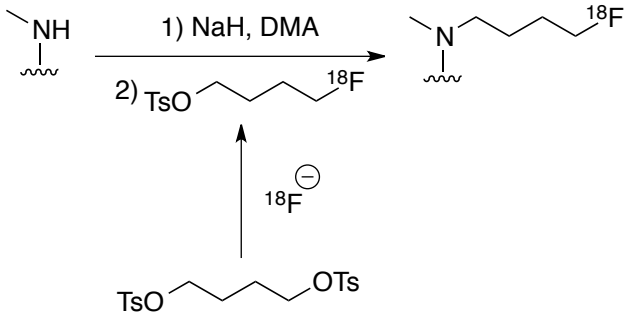


23 n = 2
24 n = 3

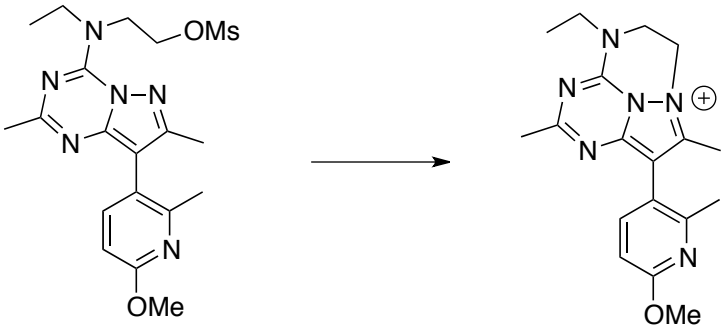
A preliminary radiolabeling was performed utilizing a [^{18}F]fluoroalkyl tosylate labeling agent derived from a ditosylate (Scheme 4). Direct labeling with a mesylate was rejected since spontaneous intramolecular cyclization to form the pyrazolo reportedly

occurs at room temperature with similar structures (Scheme 5).[18] Our methodology uses precursors from our SAR library, reducing the need to synthesize additional molecules.

Scheme 4



Scheme 5

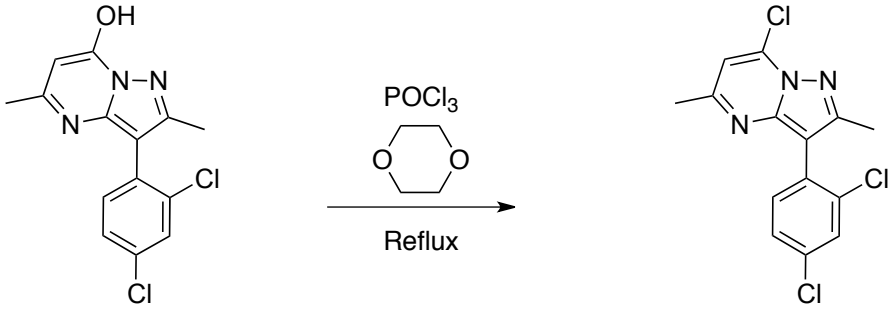


3. Experimental

General Experimental Procedures

Solvents were purchased from VWR and had originated from EMD or Burdick and Jackson. Anhydrous solvents (100-mL septum-capped bottles) were purchased from Sigma Aldrich. TLC plates used were EMD glass-backed Silica Gel 60 F_{254}, 20 x 20 cm, 250 μm. Preparatory TLC plates used were Analtech Uniplate Silica Gel GF 20 x 20 cm, 2000 μm. Silica gel used was EMD Silica Gel 60, 40-63 μm. Radial chromatography was performed with a Harrison Research Chromatotron. NMR spectrometry was performed on a Varian Mercury spectrometer at the specified frequencies.

Chemistry



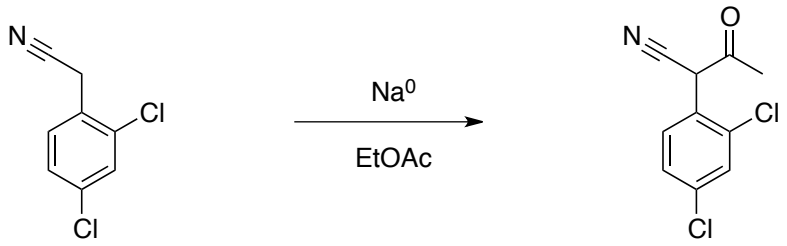
3-(2,4-dichlorophenyl)-2,5-dimethyl pyrazolo[1,5-*a*]pyrimidin-7-ol
Molecular Weight: 308.16

7-chloro-3-(2,4-dichlorophenyl)-2,5-dimethyl pyrazolo[1,5-*a*]pyrimidine
Chemical Formula: $C_{14}H_{10}Cl_3N_3$
Exact Mass: 324.99
Molecular Weight: 326.61

7-Chloro-3-(2,4-dichlorophenyl)-2,5-dimethylpyrazolo[1,5-*a*]pyrimidine (9).[19]

Compound **12** (775 mg, 2.52 mmol) was flushed with $Ar_{(g)}$ and suspended in anhydrous 1,4-dioxane (20 mL). Phosphoryl chloride (1.4 mL, 15 mmol, 6.0 eq) was added to the reaction mixture and stirred at reflux for 1 h. The solvent was removed azeotropically with heptane to give an orange-red syrup. The crude product was dissolved in CH_2Cl_2 (25

mL) and poured into ice water (50 mL). The aqueous layer was basified to pH 8-9 with conc. ammonium hydroxide. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (10 mL x 2). The organic layers were combined and dried with $MgSO_4$. The crude product was poured onto dry silica (15 cm h x 4 cm i.d.) and eluted under vacuum: Hexanes (50 mL), hexanes/CH_2Cl_2 v/v 1:1 (50 mL), 1:3 (100 mL), CH_2Cl_2 (150 mL). A second purification by vacuum flash chromatography on silica (15 cm h x 4 cm i.d.) was performed: Hexanes (100 mL), hexanes/CH_2Cl_2 v/v 3:1 (100 mL), 1:1 (100 mL), 1:3 (100 mL), CH_2Cl_2 (400 mL). Compound **9** (594 mg, 72%) was collected as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (m, 1 H), 7.34 (m, 2 H), 6.83 (s, 1 H), 2.55 (s, 3 H), 2.45 (s 3 H).

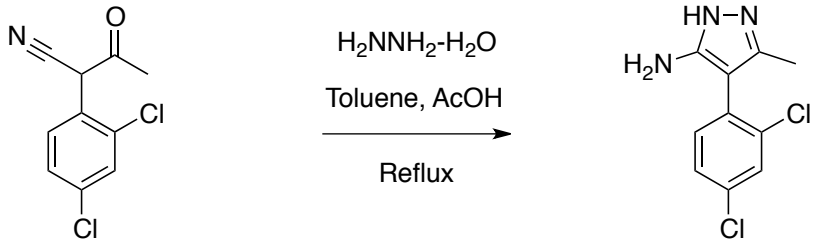


2,4-dichlorophenyl-acetonitrile
Molecular Weight: 186.04

1-cyano-1-(2,4-dichloropheyl)propan-2-one
Chemical Formula: $C_{10}H_7Cl_2NO$
Exact Mass: 226.99
Molecular Weight: 228.07

1-Cyano-1-(2,4-dichlorophenyl)propan-2-one (10).[20] 2,4-Dicholophenyl-acetonintrile (5.31 g, 28.5 mmol) was dissolved in ethyl acetate (130 mL) under $Ar_{(g)}$ followed by the addition of Na^0 (887 mg, 38.6 mmol, 1.4 eq) portionwise over 15 minutes. The solution changed from clear to pale yellow to orange. The mixture was stirred at room temperature under $Ar_{(g)}$ for 22 h. Completed reaction was pink with a white precipitate. The reaction vessel was cooled to 0°C. Precipitate was collected by vacuum filtration and washed with cold EtOAc. The precipitate was suspended in 100 mL of H_2O and acidified

with AcOH (pH 4.5). The aqueous layer was extracted with CH_2Cl_2 (100 mL x 2). The organic layers were combined and washed with H_2O (25 mL), washed with brine (50 mL), and dried over $MgSO_4$. The solvent was removed to produce **10** (4.15 g, 64%) as an off-white/yellowish solid: TLC R_f=0.42 (silica, 5% MeOH in CH_2Cl_2). The sample was used without further purification.

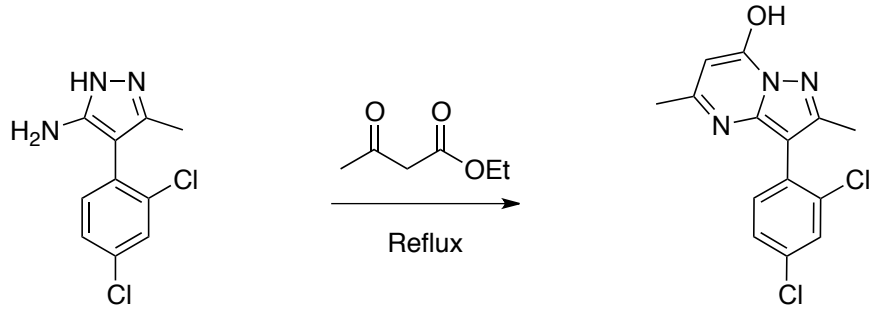


1-cyano-1-(2,4-dichloropheyl)propan-2-o
Molecular Weight: 228.07

4-(2,4-dichlorophenyl)-3-methyl-1*H*-pyrazol-5-amine
Chemical Formula: $C_{10}H_9Cl_2N_3$
Exact Mass: 241.02
Molecular Weight: 242.10

4-(2,4-Dichlorophenyl)-3-methyl-1*H*-pyrazol-5-amine (11).[20] Compound **10** (4.08 g, 17.8 mmol) was dissolved in toluene (80 mL). AcOH (2.8 mL, 49 mmol, 3 eq) and hydrazine monohydrate (1.8 mL, 37 mmol, 2 eq) were added. The flask was fitted with a Dean-stark trap and stirred at reflux under $Ar_{(g)}$ for 20 h. Water was removed from the trap every 0.5 mL. The reaction was cooled to 0°C. A gummy off-white precipitate was collected via vacuum filtration. MeOH (5 mL) was added to the filtrate and solvent was removed. Heptane and hexanes were added simultaneously to help remove residual toluene and methanol as an azeotrope. The residual yellow oil and initial precipitate were combined, dissolved in CH_2Cl_2 and H_2O, and cooled to 0°C. The pH was made 8-9 with ammonium hydroxide. The layers were separated. The aqueous layer was extracted with CH_2Cl_2 (25 mL x 2). All organic layers were combined and washed with H_2O (75 mL x 1), CH_2Cl_2 (75 mL x 1), and dried with $MgSO_4$. Removal of the solvent left impure,

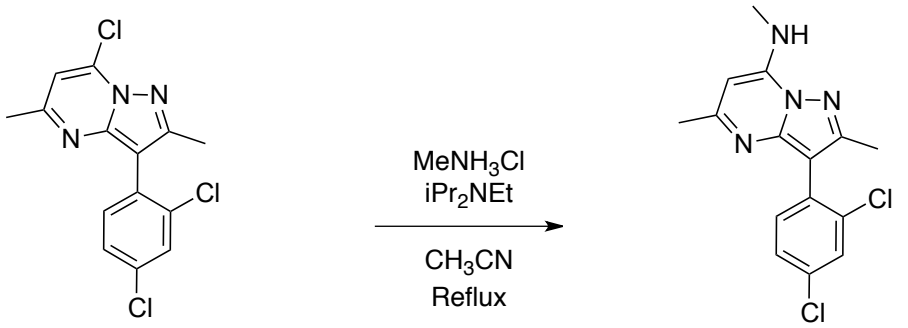
viscous, yellow oil. The crude product was dissolved in CH_2Cl_2, poured onto dry silica (15 cm x 4 cm i.d.) and eluted under vacuum: %MeOH/CH_2Cl_2 1% (100 mL), 2% (100 mL), 3% (100 mL), 4% (100 mL), 5% (600 mL). Purification by vacuum flash chromatography on silica (15 cm h x 4 cm i.d.) was repeated: 1% (100 mL), 2.5% (100 mL), 5% (600 mL) to afford **11** (2.41 g, 56%) as an off-white foam: TLC R_f=0.15 (5%MeOH in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (d, 1 H, *J*=2.1 Hz), 7.29 (dd, 1 H, *J*=2.1 Hz, *J*=8.1 Hz), 7.23 (d, 1 H, *J*=8.1 Hz), 5.30 (s, 2 H), 3.62 (br s, 1 H), 2.15 (s, 3 H).



4-(2,4-dichlorophenyl)-3-methyl-1*H*-pyrazol-5-amine
Molecular Weight: 242.10

3-(2,4-dichlorophenyl)-2,5-dimethyl pyrazolo[1,5-*a*]pyrimidin-7-ol
Chemical Formula: $C_{14}H_{11}Cl_2N_3O$
Exact Mass: 307.03
Molecular Weight: 308.16

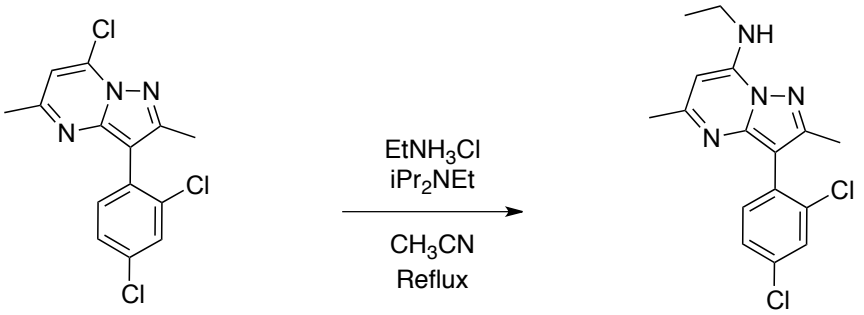
3-(2,4-Dichlorophenyl)-2,5-dimethylpyrazolo[1,5-*a*]-pyrimidin-7-ol (12).[19] Compound **11** (1.68 g, 6.94 mmol) and AcAcOEt (50 mL, 390 mmol, 57 eq) were stirred at reflux under $Ar_{(g)}$ for 46 h. The orange solution was cooled to room temperature crashing out a white precipitate. The reaction vessel was cooled in a freezer to -15°C. The precipitate was collected via vacuum filtration through a medium fritted funnel and washed with -15°C EtOAc. The precipitate was washed with room temperature hexane to yield **12** (605 mg, 28%) as a white solid.



7-chloro-3-(2,4-dichlorophenyl)-2,5-dimethyl pyrazolo[1,5-*a*]pyrimidine
Molecular Weight: 326.61

3-(2,4-dichlorophenyl)-*N*,2,5-trimethyl pyrazolo[1,5-*a*]pyrimidin-7-amine
Chemical Formula: $C_{15}H_{14}Cl_2N_4$
Exact Mass: 320.06
Molecular Weight: 321.20

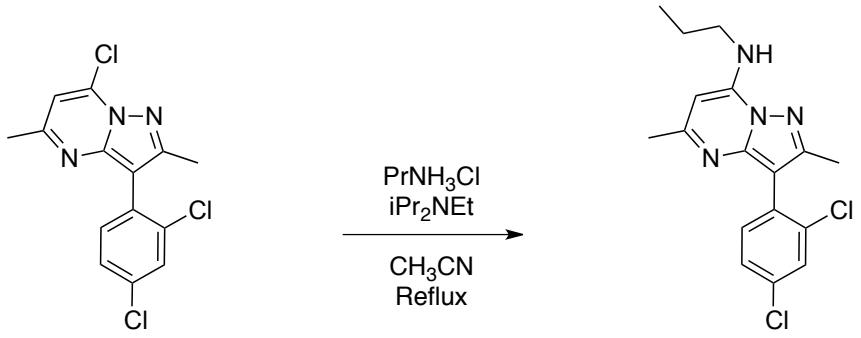
3-(2,4-Dichlorophenyl)-*N*,2,5-trimethylpyrazolo[1,5-*a*]pyrimidin-7-amine (13). Compound **9** (312 mg, 9.54 x 10^{-4} mol) and methylammonium chloride (259 mg, 3.84 mmol, 4 eq) were flushed with $Ar_{(g)}$ and dissolved in acetonitrile (20 mL). *i*Pr_2NEt was added via syringe. The reaction was stirred for 2 h. The reaction turned yellow with a white precipitate. The precipitate was isolated by filtration, dissolved in CH_2Cl_2, and eluted by vacuum on silica (15 cm h x 4 cm i.d.): hexanes (100 mL), hexanes/EtOAc/NEt_3 v/v/v 95:4:1 (100 mL), 90:8:2 (100 mL), 75:20:5 (400 mL) to afford **13** (288 mg, 94%) as a white foam: TLC R_f=0.38 (silica, hexanes/EtOAc/NEt_3 v/v/v 75:20:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (d, 1 H, *J*=2.1 Hz), 7.37 (d, 1 H, *J*=8.1 Hz), 7.30 (dd, 1 H, *J*=2.1 Hz, *J*=8.1 Hz), 6.18 (partially resolved q, 1 H), 5.81 (s, 1 H), 3.10 (d, 3 H, *J*=5.4), 2.48 (s, 3 H), 2.35 (s, 3 H).



7-chloro-3-(2,4-dichlorophenyl)-2,5-dimethyl pyrazolo[1,5-*a*]pyrimidine
Molecular Weight: 326.61

3-(2,4-dichlorophenyl)-*N*-ethyl-2,5-dimethyl pyrazolo[1,5-*a*]pyrimidin-7-amine
Chemical Formula: $C_{16}H_{16}Cl_2N_4$
Exact Mass: 334.08
Molecular Weight: 335.23

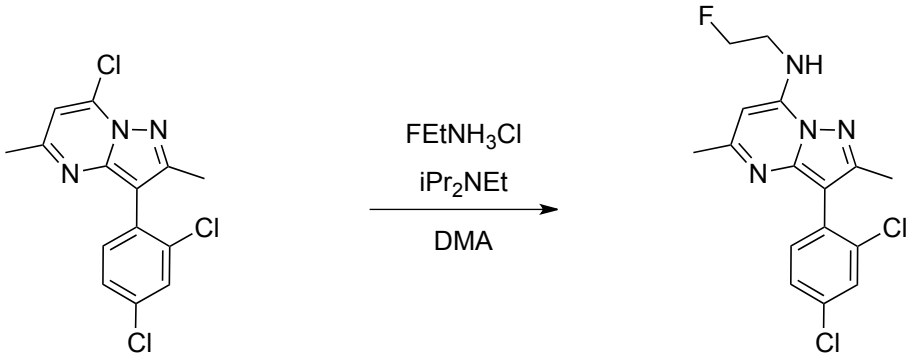
3-(2,4-Dichlorophenyl)-*N*-ethyl-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-amine (14). Compound **9** (272 mg, 8.32 x 10^{-4} mol) and ethylammonium chloride (287 mg, 3.50 mmol, 4 eq) were flushed with $Ar_{(g)}$ and dissolved in acetonitrile (20 mL). *i*Pr_2NEt was added via syringe. The reaction was stirred at reflux for 3.5 h. The crude product was dissolved in CH_2Cl_2, poured onto dry silica (15 cm x 4 cm i.d.), and eluted under vacuum: hexanes (100 mL), hexanes/EtOAc/NEt_3 v/v/v 95:4:1 (100 mL), 90:8:2 (100 mL), 75:20:5 (500 mL), 50:45:5 (100 mL). The final product was spilled on the lab bench and recovered by mopping the solution with a paper towel and extracting the paper towel with CH_2Cl_2 and eluted through a pad of silica to afford **14** (123 mg, 44%) as a white foam. TLC R_f=0.21 (silica, hexanes/EtOAc/NEt_3 v/v/v 75:20:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, 1 H, *J*=1.8 Hz), 7.37 (d, 1 H, *J*=8.4 Hz), 7.31 (dd, 1 H, *J*=1.8 Hz, *J*=8.4 Hz), 6.13 (partially resolved t, 1 H), 5.82 (s, 1 H), 3.45 (quintet, 2 H, *J*=7.2 Hz), 2.47 (s, 3 H), 2.36 (s, 3 H), 1.42 (t, 3 H, *J*=7.2 Hz).



7-chloro-3-(2,4-dichlorophenyl)-2,5-dimethyl pyrazolo[1,5-*a*]pyrimidine
Molecular Weight: 326.61

3-(2,4-dichlorophenyl)-2,5-dimethyl-*N*-propyl pyrazolo[1,5-*a*]pyrimidin-7-amine
Chemical Formula: $C_{17}H_{18}Cl_2N_4$
Exact Mass: 348.09
Molecular Weight: 349.26

3-(2,4-Dichlorophenyl)-2,5-dimethyl-*N*-propylpyrazolo[1,5-*a*]pyrimidin-7-amine (15). Compound **9** (245 mg, 7.50 x 10^{-4} mol) and propylammonium chloride (303 mg, 3.17 mmol, 4 eq) were flushed with $Ar_{(g)}$ and dissolved in acetonitrile (20 mL). *i*Pr_2NEt (1.6 mL, 9.3 mmol, 12 eq) was added via syringe. The reaction was stirred at reflux for 3 h. The solvent was removed. The crude product was dissolved in CH_2Cl_2, poured onto silica (15 cm x 4 cm i.d.), and eluted under vacuum: hexanes/EtOAc/NEt_3 v/v/v 95:4:1 (50 mL), 90:8:2 (50 mL), 75:20:5 (200 mL). Compound **15** (249 mg, 95%) was obtained as an off white foam: TLC R_f=0.37 (silica, hexanes/EtOAc/NEt_3 v/v/v 75:20:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, 1 H, *J*=2.1 Hz), 7.37 (d, 1 H, *J*=8.1 Hz), 7.31 (dd, 1 H, *J*=2.1 Hz, *J*=8.1 Hz), 6.21 (br t, 1 H, *J*=5.1 Hz), 5.82 (s, 1 H), 3.45 (q, 2 H, *J*=6.9 Hz), 2.47 (s, 3 H), 2.36 (s, 3 H), 1.78 (sextet, 2 H, *J*=6.9 Hz, *J*=7.2 Hz), 1.09 (t, 3 H, *J*=7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.1, 152.2, 147.0, 146.1, 135.8, 134.3, 133.6, 130.6, 129.8, 127.2, 105.0, 85.6, 44.0, 25.6, 22.6, 13.9, 11.7.

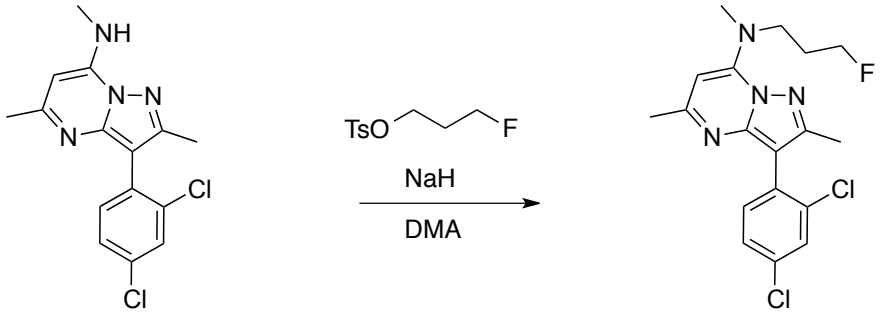


7-chloro-3-(2,4-dichlorophenyl)-2,5-dimethylpyrazolo[1,5-*a*]pyrimidine
Molecular Weight: 326.61

3-(2,4-dichlorophenyl)-*N*-(2-fluoroethyl)-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-amine
Chemical Formula: $C_{16}H_{15}Cl_2FN_4$
Exact Mass: 352.07
Molecular Weight: 353.22

3-(2,4-Dichlorophenyl)-*N*-(2-fluoroethyl)-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-amine (16). Compound **9** (135 mg, 4.13 x 10^{-4} mol) and fluoroethyl ammonium chloride (181 mg, 1.82 mmol, 4 eq) were added to a 50 mL flask and purged with $Ar_{(g)}$. The solids were dissolved in CH_3CN (15 mL) and iPr_2NEt (0.9 mL, 5 mmol, 13 eq) was added via syringe. The reaction was stirred at reflux for 24 h. The solvent was removed. The crude product was dissolved in CH_2Cl_2, poured onto dry silica (10 cm h x 4 cm i.d.), and eluted under vacuum: hexanes/EtOAc/NEt_3 v/v/v 90:8:2 (100 mL), 75:20:5 (100 mL), 50:45:5 (200 mL) afforded 111 mg of impure product. Purification by radial chromatography (1 mm silica): hexanes/EtOAc/NEt_3 v/v/v 95:4:1 (500 mL), 90:8:2 (100 mL), 75:20:5 (100 mL) afforded **16** (104 mg, 72%) was obtained as a white foam: TLC R_f=0.41 (silica, hexanes/EtOAc/NEt_3 v/v/v 75:20:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, 1 H, *J*=2.1 Hz), 7.37 (d, 1 H, *J*=8.1 Hz), 7.31 (dd, 1 H, *J*=2.1, *J*=8.1 Hz), 6.49 (br t, 1 H, *J*=5.7 Hz), 5.86 (s, 1 H), 4.72 (dt, 2 H, *J*=4.8 Hz, *J*=47.1 Hz), 3.73 (dq, 2 H, *J*=4.8 Hz, *J*=25.8 Hz), 2.48 (s, 3 H), 2.37 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.2, 152.5, 147.0, 145.8, 134.2, 133.7, 130.5, 129.8, 127.3, 105.3, 85.7, 81.8 (d, *J*=169.9 Hz), 42.5 (d, *J*=21.0 Hz),

25.6, 13.9.

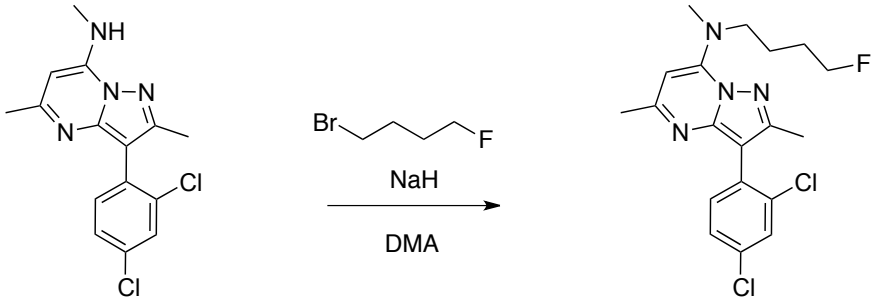


3-(2,4-dichlorophenyl)-*N*,2,5-trimethyl pyrazolo[1,5-*a*]pyrimidin-7-amine
Molecular Weight: 321.20

3-(2,4-dichlorophenyl)-*N*-(3-fluoropropyl)-*N*,2,5-trimethylpyrazolo[1,5-*a*]pyrimidin-7-amine
Chemical Formula: $C_{18}H_{19}Cl_2FN_4$
Exact Mass: 380.10
Molecular Weight: 381.27

3-(2,4-Dichlorophenyl)-*N*-(3-fluoropropyl)-*N*,2,5-trimethylpyrazolo[1,5-*a*]pyrimidin-7-amine (17). Compound **13** (76 mg, 0.24 mmol) was flushed with $Ar_{(g)}$, dissolved in anhydrous DMA (2 mL), and cooled in an ice bath to 0°C. NaH (60%) (21 mg, 8.78 x 10^{-4} mol, 4 eq) was added and allowed to stir under $Ar_{(g)}$ until bubbling ceased (10 min). After adding **24** (289 mg, 1.24 mmol, 5 eq), the reaction was warmed to room temperature and stirred for 21 h. The brown reaction mixture was poured into H_2O (25 mL) and extracted with EtOAc (10 mL, 5mL x 3). The organic layers were combined, washed with H_2O (5 mL x 3), and dried with $MgSO_4$. Removal of the solvent left a yellow oil. The crude product was dissolved in CH_2Cl_2, poured onto silica (10 cm h x 4 cm i.d.) and eluted by vacuum: hexanes (100 mL), hexanes/EtOAc/NEt_3 v/v/v 95:4:1 (150 mL), 90:8:2 (200 mL), 75:20:5 (200 mL). Purification by radial chromatography (2 mm): 95:4:1 (250 mL), 90:8:2 (250 mL), 75:20:5 (100 mL), followed by purification by vacuum flash chromatography (10 cm h x 4 cm i.d.): CH_2Cl_2 (400 mL), 75:20:5 (600 mL), and followed by purification by column chromatography (6 cm h x 1 cm i.d.): hexanes/EtOAc/NEt_3 v/v/v 90:8:2 (200 mL) afforded **17** (21 mg, 24%) as a clear oil: TLC

R_f=0.25 (silica, hexanes/EtOAc/NEt_3 v/v/v 75:20:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, 1 H, *J*=2.1 Hz), 7.36 (d, 1 H, *J*=8.4 Hz), 7.30 (dd, 1 H, *J*=2.1 Hz, *J*=8.4 Hz), 5.87 (s, 1 H), 4.60 (dt, 2 H, *J*=5.7 Hz, $^1J_{HF}$=47.1 Hz), 4.08 (br t, 2 H, *J*=6.6 Hz), 3.21 (s, 3 H), 2.46 (s, 3 H), 2.35 (s, 3 H), 2.20 (d quintet, 2 H, *J*=6.6 Hz, $^2J_{HF}$=26.7 Hz).

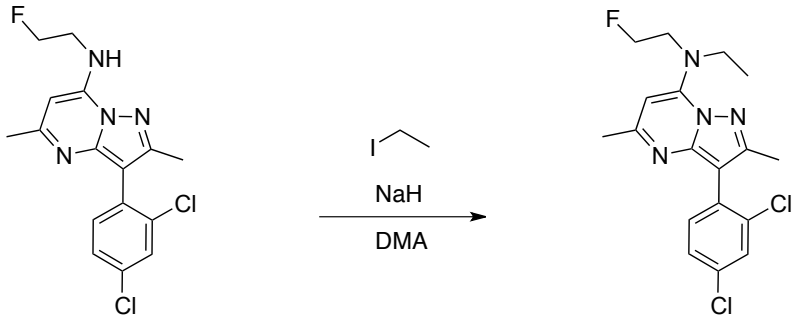


3-(2,4-dichlorophenyl)-*N*,2,5-trimethyl pyrazolo[1,5-*a*]pyrimidin-7-amine
Molecular Weight: 321.20

3-(2,4-dichlorophenyl)-*N*-(4-fluorobutyl)-*N*,2,5-trimethyl pyrazolo[1,5-*a*]pyrimidin-7-amine
Chemical Formula: $C_{19}H_{21}Cl_2FN_4$
Exact Mass: 394.11
Molecular Weight: 395.30

3-(2,4-Dichlorophenyl)-*N*-(4-fluorobutyl)-*N*,2,5-trimethylpyrazolo[1,5-*a*]pyrimidin-7-amine (18). Compound **13** (141 mg, 4.38 x 10^{-4} mol) was flushed with $Ar_{(g)}$, dissolved in anhydrous DMA (2.5 mL), and cooled to 0°C. NaH (60%) (44 mg, 1.1 mmol, 2.5 eq) was added and allowed to stir under $Ar_{(g)}$ until bubbling ceased (10 min). After adding 1-bromo-4-fluorobutane (0.25 mL, 2.2 mmol, 5 eq), the reaction was warmed to room temperature and stirred for 22 h. The brown reaction mixture was poured into H_2O (25 mL) and extracted with EtOAc (10 mL, 5mL x 3). The organic layers were combined, washed with H_2O (5 mL x 3), and dried with $MgSO_4$. Removal of the solvent left a yellow oil. The crude product was dissolved in CH_2Cl_2, poured onto silica (10 cm h x 4 cm i.d.) and eluted by vacuum: hexanes (50 mL), hexanes/EtOAc/NEt_3 v/v/v 90:8:2 (100 mL), 75:20:5 (200 mL). Purification by radial chromatography (1mm silica):

hexanes/EtOAc/NEt_3 v/v/v 95:4:1 (300 mL) afforded compound **18** (82 mg, 47%) as a white solid. TLC R_f=0.34 (silica, hexanes/EtOAc/NEt_3 v/v/v 75:20:5); ^{1}H NMR (300 Hz, $CDCl_3$) δ 7.52 (d, 1 H, *J*=2.1 Hz), 7.37 (d, 1 H, *J*=8.4 Hz), 7.31 (dd, 1 H, *J*=8.4 Hz, *J*=2.1 Hz), 5.85 (s, 1 H), 4.52 (dt, 2 H, *J*=5.7 Hz, $^1J_{HF}$=47.4 Hz), 4.02 (br s, 2 H), 3.18 (s, 3 H), 2.45 (s, 3 H), 2.36 (s, 3 H), 1.85 (overlapping m dm, 3 H+1 H), 1.73 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.5, 151.9, 149.5, 149.2, 135.7, 134.3, 133.5, 130.8, 129.7, 127.2, 104.4, 92.3, 83.9 (d, *J*=83.9 Hz), 52.3, 38.9, 27.8 (d, 20.0 Hz), 25.2, 23.9 (d, *J*=4.5 Hz), 14.0.

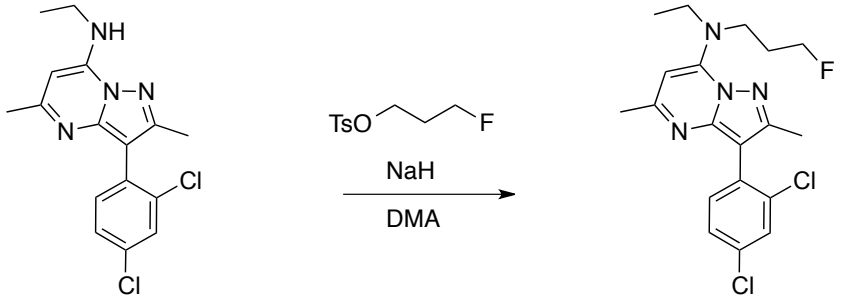


3-(2,4-dichlorophenyl)-*N*-(2-fluoroethyl)-2,5-dimethyl pyrazolo[1,5-*a*]pyrimidin-7-amine
Molecular Weight: 353.22

3-(2,4-dichlorophenyl)-*N*-ethyl-*N*-(2-fluoroethyl)-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-amine
Chemical Formula: $C_{18}H_{19}Cl_2FN_4$
Exact Mass: 380.10
Molecular Weight: 381.27

3-(2,4-Dichlorophenyl)-*N*-ethyl-*N*-(2-fluoroethyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-7-amine (19). Compound **16** (53 mg, 0.15 mmol) was flushed with $Ar_{(g)}$, dissolved in anhydrous DMA (2 mL), and cooled in an ice bath to 0°C. NaH (60%) (23 mg, 0.56 mmol, 4 eq) was added and allowed to stir under $Ar_{(g)}$ until bubbling ceased (10 min). After adding iodoethane (60 μL, 1.5 mmol, 5 eq), the reaction was warmed to room temperature and stirred for 25 h. The brown reaction mixture was poured into H_2O (20 mL) and extracted with EtOAc (10 mL, 5mL x 3). The organic layers were combined,

washed with H_2O (5 mL x 3), and dried with $MgSO_4$. Removal of the solvent left a yellow oil. The crude product was dissolved in CH_2Cl_2, poured onto silica (12 cm h x 4 cm i.d.) and eluted by vacuum: hexanes/EtOAc/NEt_3 v/v/v 95:4:1 (200 mL), 90:8:2 (200 mL), 75:20:5 (200 mL). Purification by radial chromatography (1 mm silica): hexanes/EtOAc/NEt_3 v/v/v 98:1:1 (300 mL) afforded compound **19** (30 mg, 53%) as a white solid: TLC R_f=0.36 (silica, hexanes/EtOAc/NEt_3 v/v/v 75:20:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, 1 H, *J*=1.8 Hz), 7.36 (d, 1 H, *J*=8.4 Hz), 7.30 (dd, 1 H, *J*=8.4 Hz, *J*=1.8 Hz), 5.93 (s, 1 H), 4.82 (dt, 2 H, *J*=5.1 Hz, $^1J_{HF}$=42.6 Hz), 4.31 (br d, 2 H, $^2J_{HF}$=24.6 Hz), 3.71 (quartet, 2 H, *J*=6.9 Hz), 2.46 (s, 3 H), 2.33 (s, 3H), 1.35 (t, 3 H, *J*=6.9 Hz); ^{13}C (75 MHz, $CDCl_3$) δ 159.5, 151.9, 149.4, 148.3, 135.7, 134.3, 133.6, 130.7, 129.8, 127.2, 104.4, 92.4, 84.6 (d, *J*=167.6 Hz), 50.7 (d, *J*= 20.0 Hz), 47.1, 25.3, 14.1, 12.3.

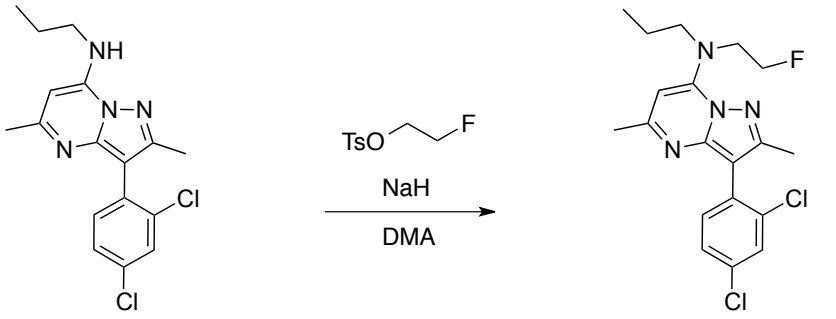


3-(2,4-dichlorophenyl)-*N*-ethyl-2,5-dimethyl pyrazolo[1,5-*a*]pyrimidin-7-amine
Molecular Weight: 335.23

3-(2,4-dichlorophenyl)-*N*-ethyl-*N*-(3-fluoropropyl)-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-amine
Chemical Formula: $C_{19}H_{21}Cl_2FN_4$
Exact Mass: 394.11
Molecular Weight: 395.30

3-(2,4-Dichlorophenyl)-*N*-ethyl-*N*-(3-fluoropropyl)-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-amine (20). Compound **14** (44 mg, 0.13 mmol) was purged with $Ar_{(g)}$, dissolved in anhydrous DMA (2 mL), and cooled to 0°C. NaH (60%) (11 mg, 0.45 mmol, 3.5 eq) was added and allowed to stir under $Ar_{(g)}$ until bubbling ceased (10 min). After

adding **24** (159 mg, 6.82 x 10^{-4} mol, 5 eq) via syringe, the reaction was warmed to room temperature and stirred for 18 h. The brown reaction mixture was poured into H_2O (25 mL) and extracted with EtOAc (10 mL, 5 mL x 3). The organic layers were combined, washed with H_2O (5 mL x 3), and dried with $MgSO_4$. Removal of the solvent left a yellow oil. The crude product was dissolved in CH_2Cl_2, poured onto dry silica (10 cm h x 4 cm i.d.), and eluted under vacuum: hexanes (100 mL), hexanes/EtOAc/NEt_3 v/v/v 95:4:1 (200 mL), 90:8:2 (200 mL), 75:20:5 (200 mL). Purification was repeated by radial chromatography (1 mm silica): hexanes/EtOAc/NEt_3 v/v/v 95/4/1 (250 mL), 90:8:2 (50 mL). Purification was repeated by radial chromatography (1mm silica): hexanes/EtOAc/NEt_3 v/v/v 98:1:1 (200 mL), 75:20:5 (25 mL). Compound **20** still contains impurities.

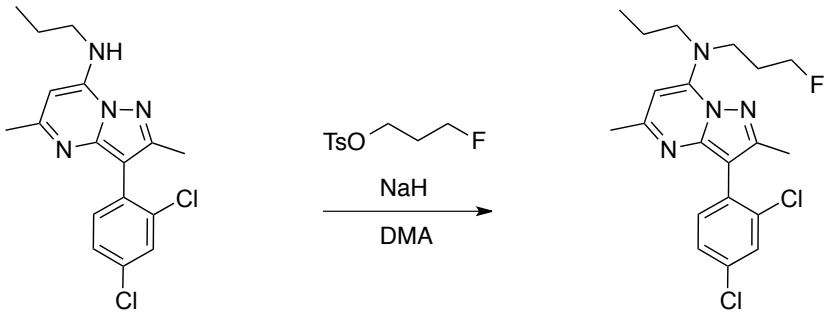


3-(2,4-dichlorophenyl)-2,5-dimethyl-*N*-propyl pyrazolo[1,5-*a*]pyrimidin-7-amine
Molecular Weight: 349.26

3-(2,4-dichlorophenyl)-*N*-(2-fluoroethyl)-2,5-dimethyl-*N*-propylpyrazolo[1,5-*a*]pyrimidin-7-amine
Chemical Formula: $C_{19}H_{21}Cl_2FN_4$
Exact Mass: 394.11
Molecular Weight: 395.30

3-(2,4-dichlorophenyl)-*N*-(2-fluoroethyl)-2,5-dimethyl-*N*-propylpyrazolo[1,5-*a*]pyrimidin-7-amine (21). Compound **15** (91 mg, 0.26 mmol) was flushed with $Ar_{(g)}$, dissolved in anhydrous DMA (2 mL), and cooled to 0°C. NaH (60%) (35 mg, 0.88 mmol, 3 eq) was added and allowed to stir under $Ar_{(g)}$ until bubbling ceased (10 min). After

adding **22** (282 mg, 1.29 mmol, 5 eq), the reaction was warmed to room temperature and stirred for 26 h. The brown reaction mixture was poured into H_2O (20 mL) and extracted with EtOAc (10 mL, 5mL x 3). The organic layers were combined, washed with H_2O (5 mL x 3), and dried with $MgSO_4$. Removal of the solvent left a yellow oil. The crude product was dissolved in CH_2Cl_2, poured onto silica (4 cm h x 4 cm i.d.) and eluted by vacuum: hexanes (50 mL), hexanes/EtOAc/NEt_3 v/v/v 95:4:1 (100 mL), 90:8:2 (100 mL), 75:20:5 (250 mL), 50:45:5 (50 mL). Purification by radial chromatography (1 mm silica): hexanes/EtOAc/NEt_3 v/v/v 95:4:1 (300 mL), 90:8:2 (50 mL) afforded an impure yellow oil. Purification by column chromatography (12 cm h, 1 cm i.d.): hexanes/EtOAc/NEt_3 v/v/v 90:8:2 afforded impure product. After another purification by radial chromatography (1 cm): hexanes/EtOAc/NEt_3 v/v/v 98:1:1 (200 mL) afforded crude **21** as a yellow residue.



3-(2,4-dichlorophenyl)-2,5-dimethyl-*N*-propyl pyrazolo[1,5-*a*]pyrimidin-7-amine
Molecular Weight: 349.26

3-(2,4-dichlorophenyl)-*N*-(3-fluoropropyl)-2,5-dimethyl-*N*-propylpyrazolo[1,5-*a*]pyrimidin-7-amine
Chemical Formula: $C_{20}H_{23}Cl_2FN_4$
Exact Mass: 408.13
Molecular Weight: 409.33

3-(2,4-dichlorophenyl)-*N*—(3-fluoropropyl)-2,5-dimethyl-*N*-propylpyrazolo[1,5-*a*]pyrimidin-7-amine (22). Compound **15** (76 mg, 0.22 mmol) was flushed with $Ar_{(g)}$, dissolved in anhydrous DMA (2 mL), and cooled to 0°C. NaH (60%) (36 mg, 0.89 mmol,

4 eq) was added and allowed to stir under $Ar_{(g)}$ until bubbling ceased (10 min). After adding **24** (242 mg, 1.04 mmol, 5 eq), the reaction was warmed to room temperature and stirred for 23 h. The brown reaction mixture was poured into H_2O (20 mL) and extracted with EtOAc (10 mL, 5mL x 3). The organic layers were combined, washed with H_2O (5 mL x 3), and dried with $MgSO_4$. Removal of the solvent left a yellow oil. The crude product was dissolved in CH_2Cl_2, poured onto silica (6 cm h x 4 cm i.d.) and eluted by vacuum: hexanes (50 mL), hexanes/EtOAc/NEt_3 v/v/v 95:4:1 (100 mL), 90:8:2 (100 mL), 75:20:5 (250 mL). Purification by radial chromatography (1 mm silica): hexanes/EtOAc/NEt_3 v/v/v 95:4:1 (300 mL) afforded a yellow oil. Purification by radial chromatography (1 mm silica): hexanes/EtOAc/NEt_3 v/v/v 98:1:1 (150 mL) afforded a yellow oil. Purification by radial chromatography (1 mm silica): hexanes/EtOAc/NEt_3 v/v/v 98:1:1 (150 mL) afforded a yellow residue. Purification by preparatory TLC 75:20:5 afforded impure **22** as a yellow residue.

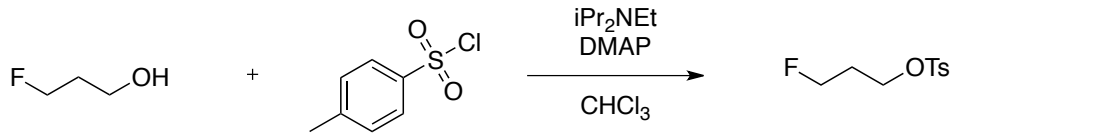
2-fluoroethanol
Molecular Weight: 64.06

Tosyl Chloride
Molecular Weight: 190.65

2-fluoroethyl 4-methylbenzenesulfonate
Chemical Formula: $C_9H_{11}FO_3S$
Exact Mass: 218.04
Molecular Weight: 218.25

2-Fluoroethyl tosylate (23). 2-Fluoroethanol (1.2 mL, 20 mmol), tosyl chloride (4.57 g, 24.0 mmol, 1.2 eq), *i*Pr_2NEt (4.3 mL, 25 mmol, 1.2 eq), and 4-dimethylaminopyridine (502 mg, 4.11 mmol, 0.2 eq) were dissolved in chloroform (50 mL) and stirred under $Ar_{(g)}$ for 93 h. The solvent was removed by rotary evaporator to give a yellow oil. The crude product was dissolved in CH_2Cl_2, poured onto silica (10 cm

h x 4 cm i.d.), and eluted by vacuum: hexanes/CH_2Cl_2 v/v 1:1 (200 mL), 1:3 (200 mL), CH_2Cl_2 (200 mL). Compound **23** (1.80 g, 40%) was afforded as an oil: TLC R_f=0.45 (silica, CH_2Cl_2): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, 2 H, *J*=8.1 Hz), 7.36 (d, 2 H, *J*=8.1 Hz), 4.57 (dt, 2 H, *J*=4.2 Hz, $^1J_{HF}$=47.1 Hz), 4.26 (dt, 2 H, *J*=4.2 Hz, $^2J_{HF}$=27.0 Hz), 2.45 (s, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.4, 132.6, 130.1, 128.1, 80.7 (d, *J*=172.5 Hz), 68.7 (d, *J*=20.5 Hz), 21.7.



3-fluoropropan-1-ol
Molecular Weight: 78.09

Tosyl Chloride
Molecular Weight: 190.65

3-fluoropropyl 4-methylbenzenesulfonate
Chemical Formula: $C_{10}H_{13}FO_3S$
Exact Mass: 232.06
Molecular Weight: 232.27

3-Fluoropropyl tosylate (24). Tosyl chloride (1.26 g, 6.62 mmol, 1.2 eq) and 4-dimethylaminopyridine (128 mg, 1.05 mmol, 0.2 eq) were flushed with $Ar_{(g)}$ and dissolved in chloroform (15 mL). 3-fluoropropanol (433 g, 5.54 mmol) and iPr_2NEt (1.3 mL, 7.2 mmol, 1.3 eq) were added to the reaction mixture. The reaction mixture was stirred at room temperature for 68 h. The solvent was removed. The crude product was dissolved in CH_2Cl_2, poured onto dry silica (10 cm h x 4 cm i.d.), and eluted under vacuum: hexanes/CH_2Cl_2 v/v 1:1 (200 mL), 1:3 (200 mL), CH_2Cl_2 (200 mL). Compound **24** (854 mg, 66%) was afforded as an oil and was used without further purification. TLC R_f=0.45 (silica, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, 2 H, *J*=8.4 Hz), 7.36 (d, 2H, *J*=8.4 Hz), 4.42 (dt, 2 H, *J*=6.0 Hz, $^1J_{HF}$=46.8 Hz), 4.17 (t, 2 H, *J*=6.0 Hz), 2.46 (s, 3 H), 2.04 (d quintet, 2 H, *J*=6.0 Hz, $^2J_{HF}$=26.1 Hz).

Binding Studies

CRF-1 receptor binding assays were performed in the Neuropsychopharmacology Laboratory, Department of Psychiatry and Behavioral Sciences, Emory University. HEK293T cells were transfected with plasmids containing the human CRF-1 receptor. Using the transfected cells, competition binding assays were performed between the candidate ligands and [^{125}I]sauvagine (Perkin Elmer). A one-site competition nonlinear regression curve analysis (GraphPad Prism 4.0; GraphPad Software, La Jolla, CA) was performed to determine the equilibrium binding constants with the concentration of [^{125}I]sauvagine set at 80 pM and K_d = 0.5 nM.

4. Results and Discussion

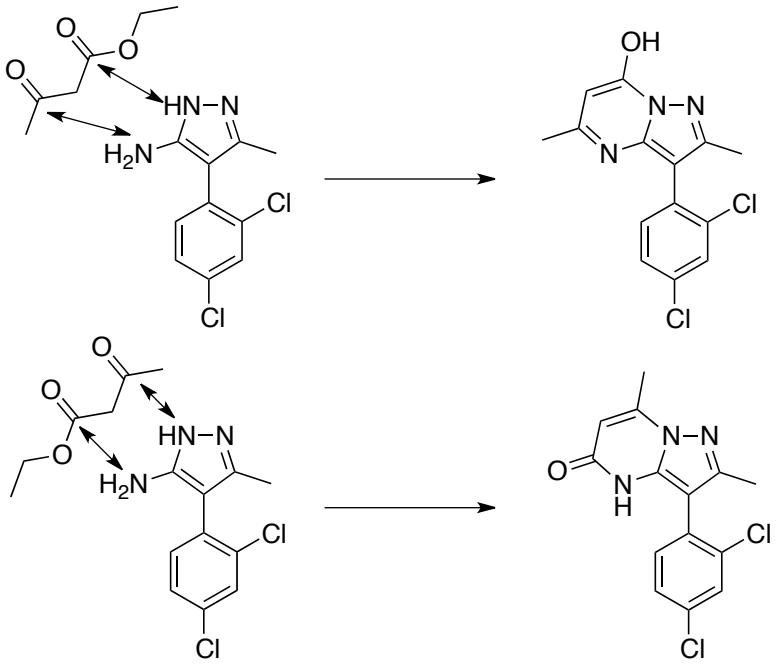
Table 1. Core Construction Yields

Compound	Yield
10	64%
11	56%
12	28%
9	72%

Compound **9** was synthesized in four steps from commercially available products with good yields (Table 1). A low yield is expected for the formation of **12** since two isomers are possible depending on which carbonyl group of AcAcOEt reacts with the primary or secondary amine (Scheme 6). Fortunately, the desired isomer precipitates out of the solution and can be isolated by filtration (confirmed by an x-ray crystal structure of **9** obtained by Dr. Stehouwer). The literature reports the use of acid and toluene for this reaction; however, we were able to force the reaction under neutral conditions by refluxing **11** in AcAcOEt (bp=185°C).

The 3-(2,4-dichlorophenyl)-*N*-alkyl-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-amines (**13 -16**) were synthesized in high yields (Table 2). A poor yield for **14** is due to human error, when the final solution was spilled on the lab bench. Mopping the spilt solution with a paper towel and extracting into CH_2Cl_2 salvaged some of the product.

Scheme 6



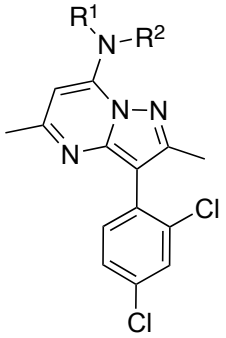
SAR analysis of molecules **13**, **14**, and **15** (Table 2) shows that binding affinity to CRF_1 directly correlates to alkyl chain length. Increasing the chain length from methyl to ethyl, increases binding by an order of magnitude. Subsequent methylene additions have less dramatic of an effect, only halving the K_i from ethyl to propyl.

While the secondary 7-amines are not cold versions of potential tracers, these molecules are potential metabolites from N-dealkylation of a radiotracer. These binding assays are necessary to determine if the potential metabolites would interfere with radiotracer binding.

The *N*-alkyl-3-(2,4-dichlorophenyl)-*N*-fluoroalkyl-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-amines (**17 - 22**) were formed using sodium hydride and either a halofluoroalkane (**18)** a haloalkane **(19**), or fluoroalkyl tosylate in DMA. Attempts were made to synthesize **18** with potassium carbonate and acetonitrile, an easier system to work with because the base is more water tolerant and the solvent is more easily

removed. Unfortunately, after two days of refluxing, only starting material was recovered. Sodium hydride in DMA was used for the remaining molecules.

Table 2. Binding Affinities and Yields of Target Compounds



Compound	R_1	R_2	K_i (nM)	Yield
13	Me	H	33	94%
14	Et	H	3.0	44%
15	Pr	H	1.6	95%
16	FEt	H	*	72%
17	Me	FPr	*	24%
18	Me	FBu	0.72	47%
19	Et	FEt	*	53%
20	Et	FPr	*	ND
21	Pr	FEt	*	ND
22	Pr	FPr	*	ND

*=binding affinities not yet determined, ND= not determined

Compound **19** was synthesized using **16** as a precursor. While adding the fluoroethyl moiety prior to the ethyl moiety is not conducive for radiolabeling, purification of this molecule was simpler than the other molecules, requiring only one purification by radial chromatography.

Compound **18** is the only fluorinated target compound that has been assessed to date by the Owens group in the Psychiatry department for binding. As predicted by its increased lipophilicity, the molecule binds strongly to CRF_1. Structures with yields reported but lacking binding data are currently being assayed.

Structures reported without yields have been synthesized but, according to NMR spectra (Appendix I), are still impure after numerous attempts at purification utilizing various solvent systems and techniques. If purification continues to be difficult, the fluoroalkyl precursors may be made. Future radiolabeling would use the current methodology since purification is conducted by reverse phase HPLC.

Preliminary attempts to synthesize [^{18}F]**18** have been successful (experiments conducted in conjunction with Dr. Stehouwer). Radioactive purity is established by an HPLC analysis. Low concentrations of radiotracer are not registered by UV detectors but are visible by radiation detectors. A co-injection of the cold (non-radioactive) product and radiotracer establish that the correct product was isolated. UV detectors detect the cold compound while a radiation detector detects the radiotracer. Slight retention time shifts are due to the sequence of detectors (Appendix II). This is proof of concept that the synthetic scheme is amenable for radiolabeling.

5. Conclusions

N-alkyl-3-(2,4-dichlorophenyl)-*N*-fluoroalkyl-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-amines have been successfully synthesized and verified by NMR spectroscopy. Preliminary binding data suggests that this class of molecules will bind strongly to CRF_1. Initial studies have been carried out to suggest that these molecules can be labeled with ^{18}F.

Future work will involve purifying the remaining compounds. Pending the completion of binding assays, the most promising candidates will be labeled with ^{18}F. Successful labeling will enable log *P* assessments, biodistribution studies in rats, and possible microPET studies in non-human primates.

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6. References

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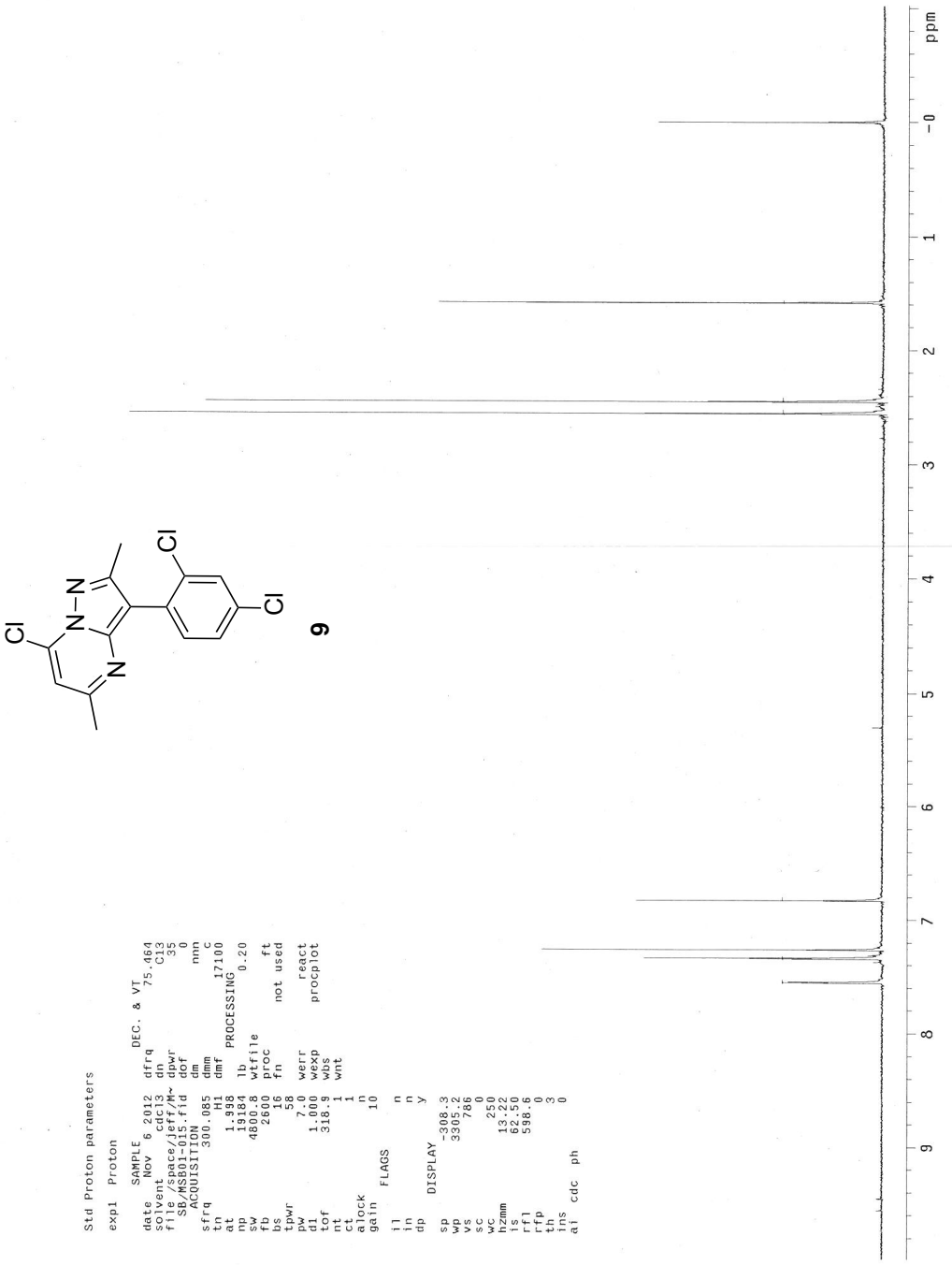
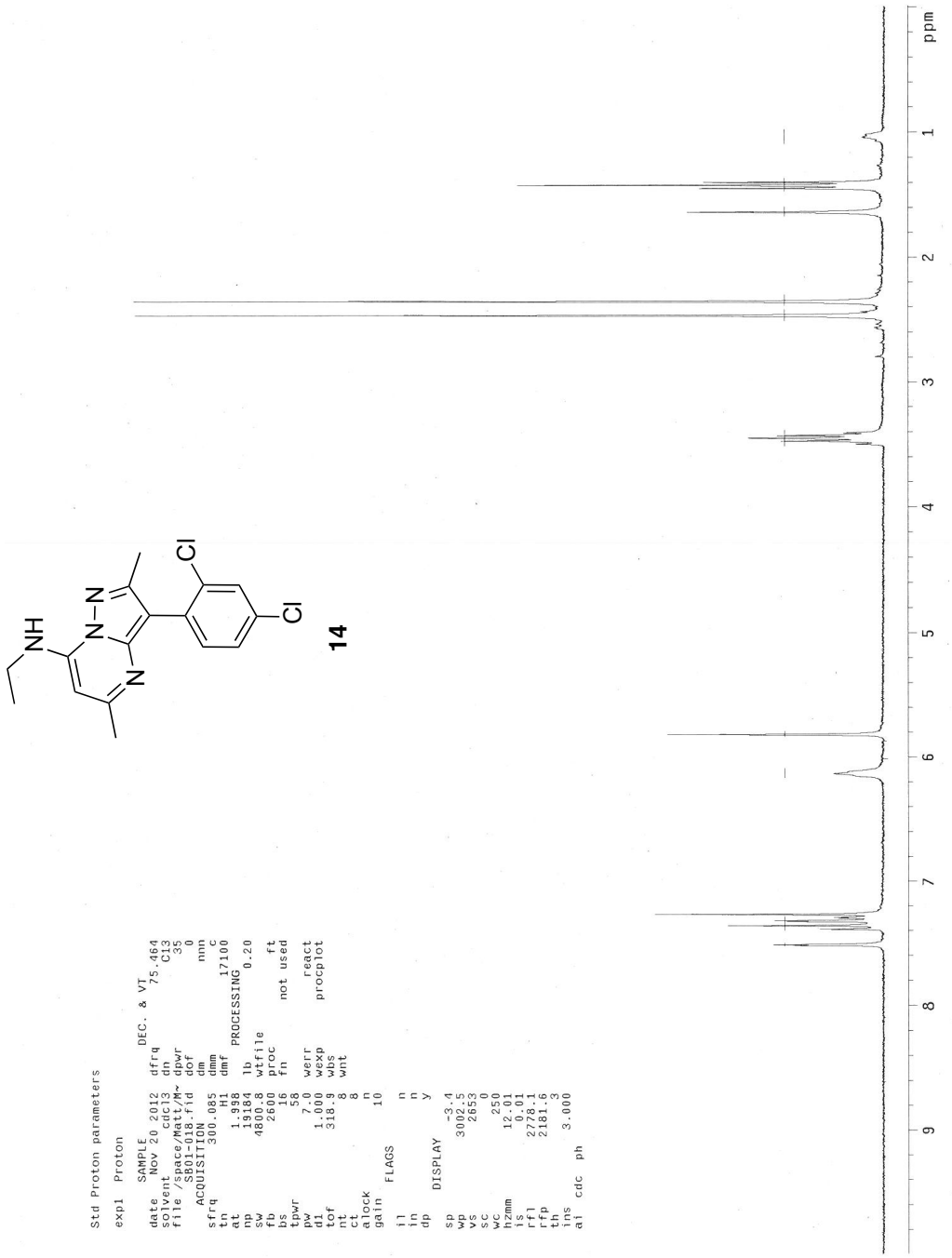
Appendix I. NMR Spectra



Spectrum 1. ^{1}H NMR Spectrum (300 MHz, $CDCl_3$) of Compound **9**

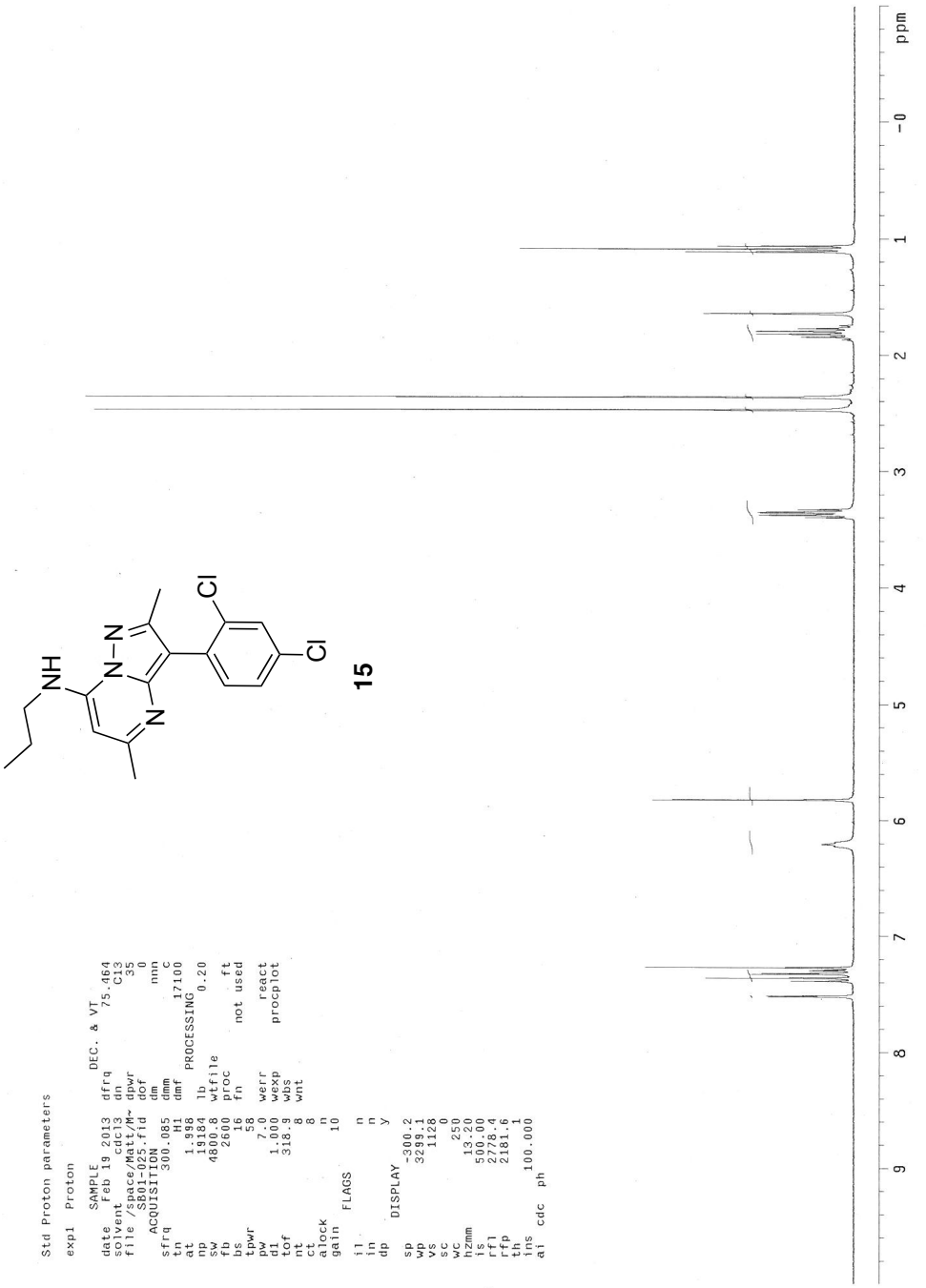
b

Spectrum 2. ^{1}H NMR Spectrum (300 MHz, $CDCl_3$) of Compound **13**



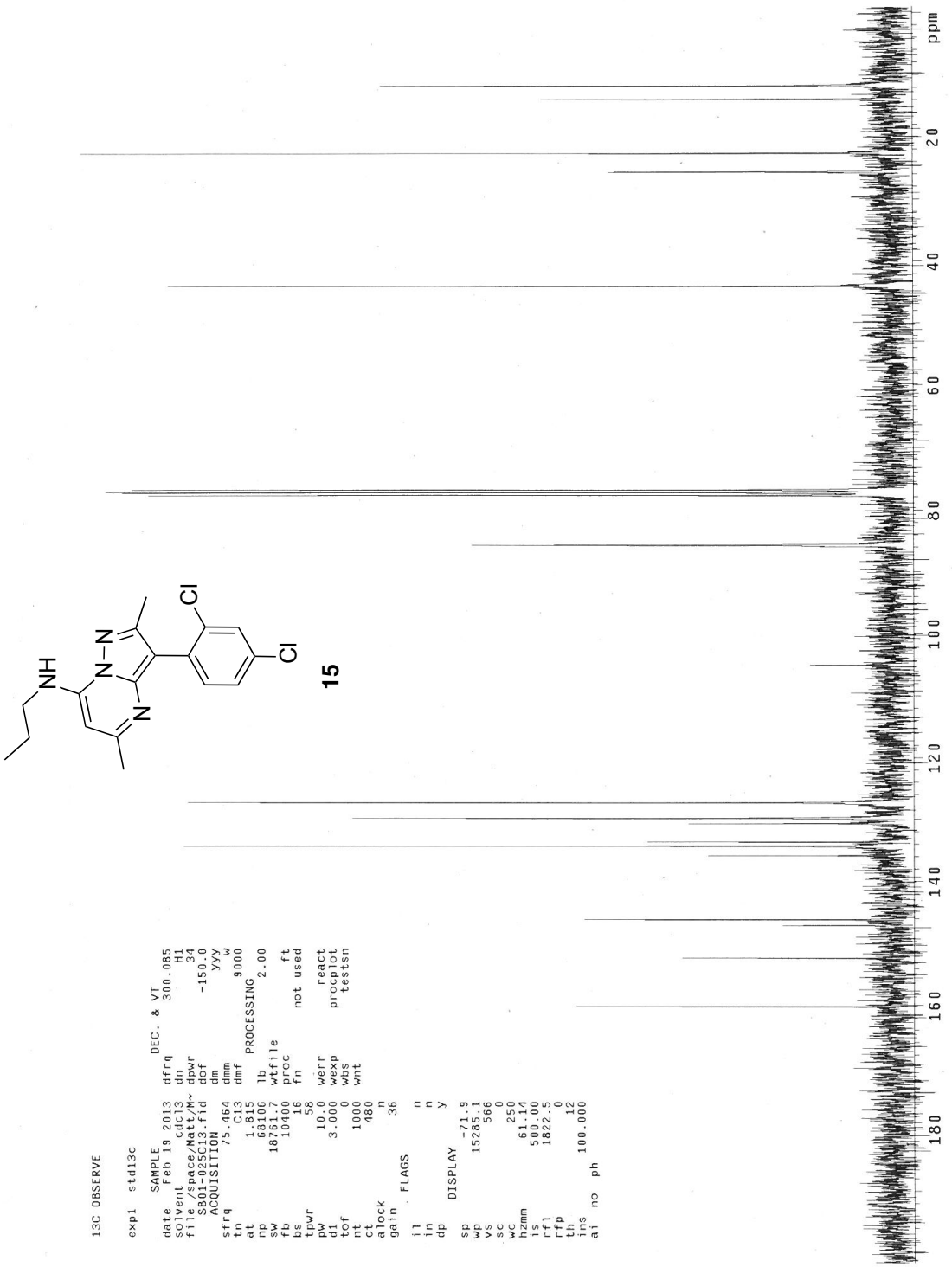
Spectrum 3. ^{1}H NMR Spectrum (300 MHz, $CDCl_3$) of Compound **14**

d

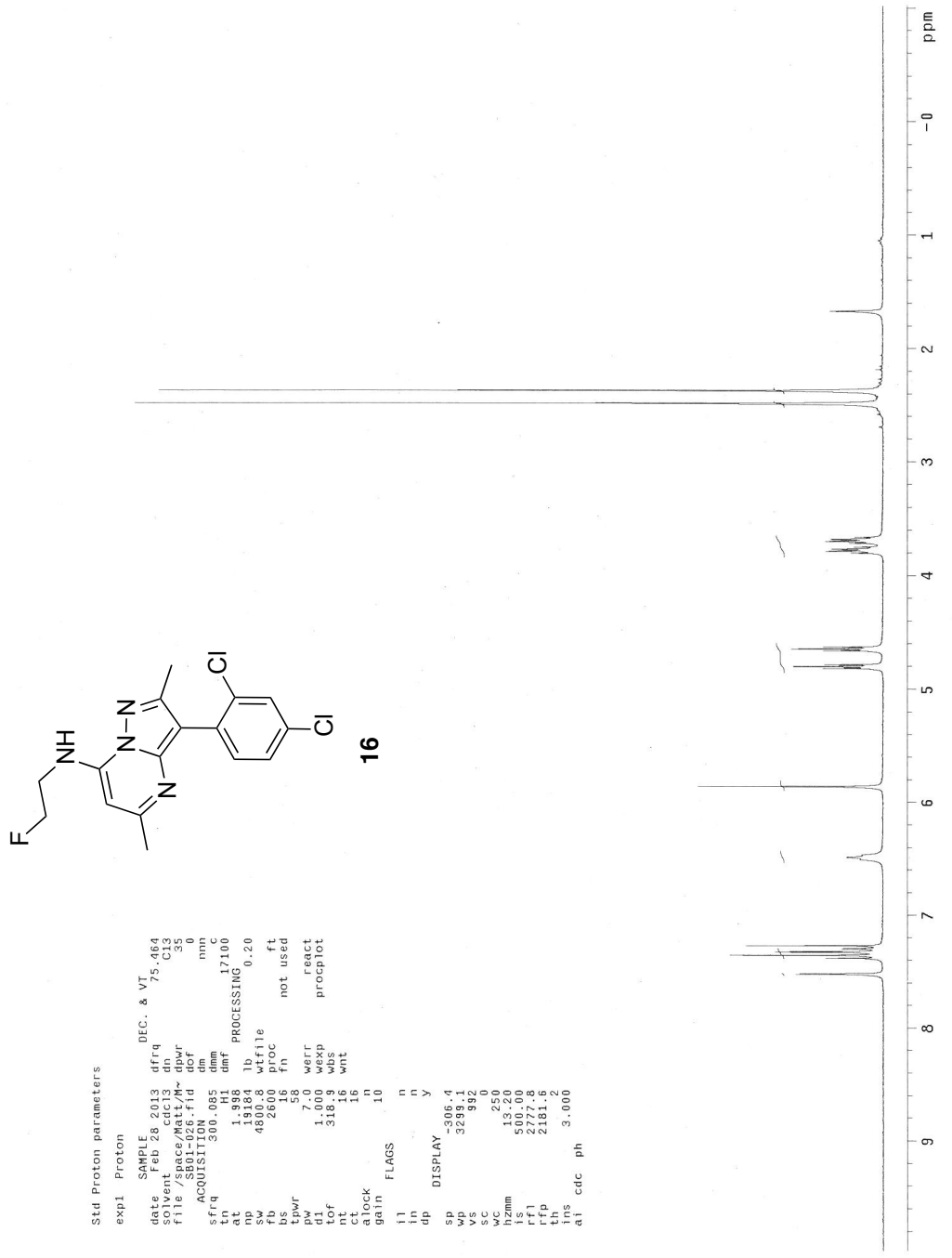


Spectrum 4. ^{1}H NMR Spectrum (300 MHz, $CDCl_3$) of Compound **15**

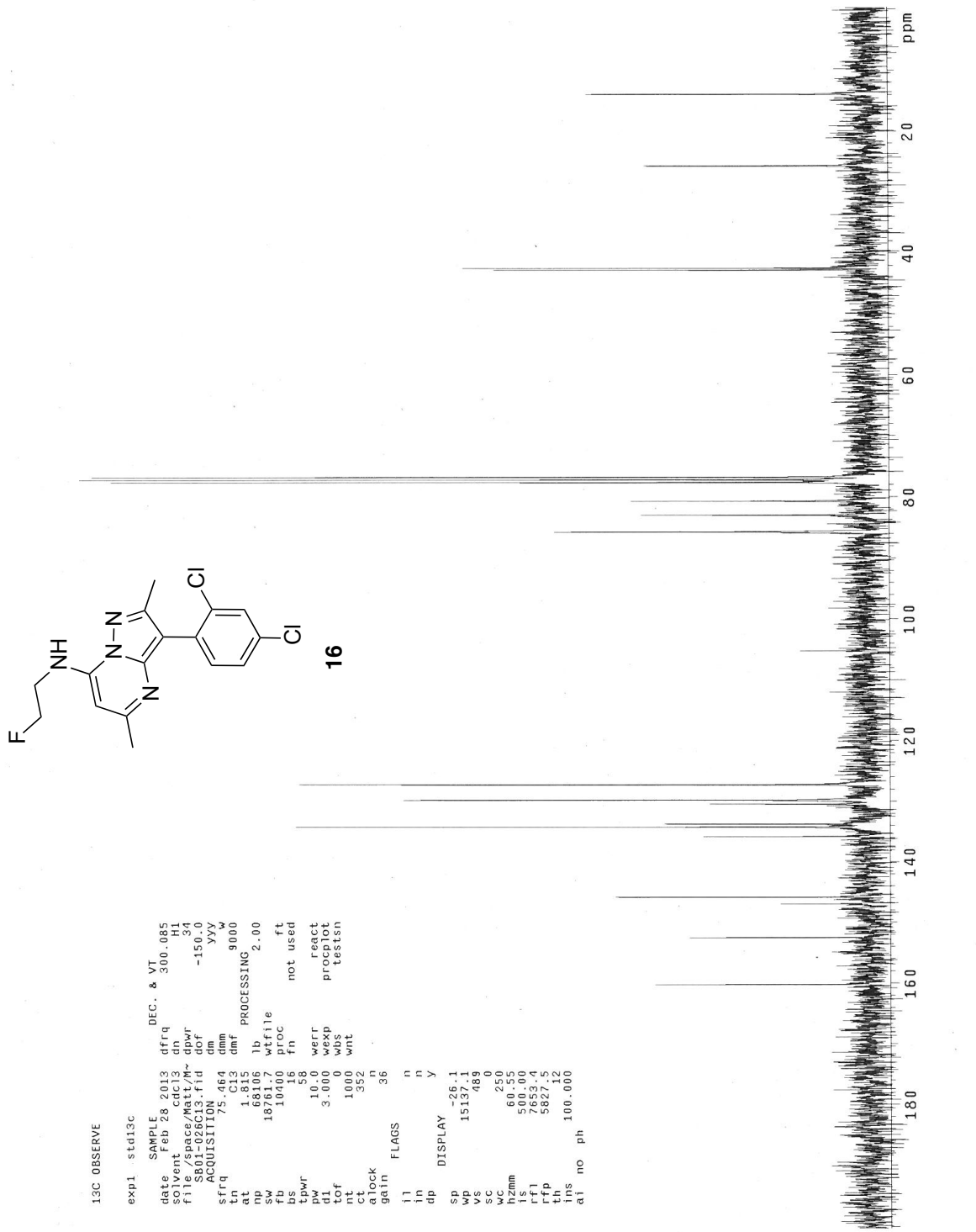
e



Spectrum 5. ^{13}C NMR Spectrum (75 MHz, $CDCl_3$) of Compound **15**



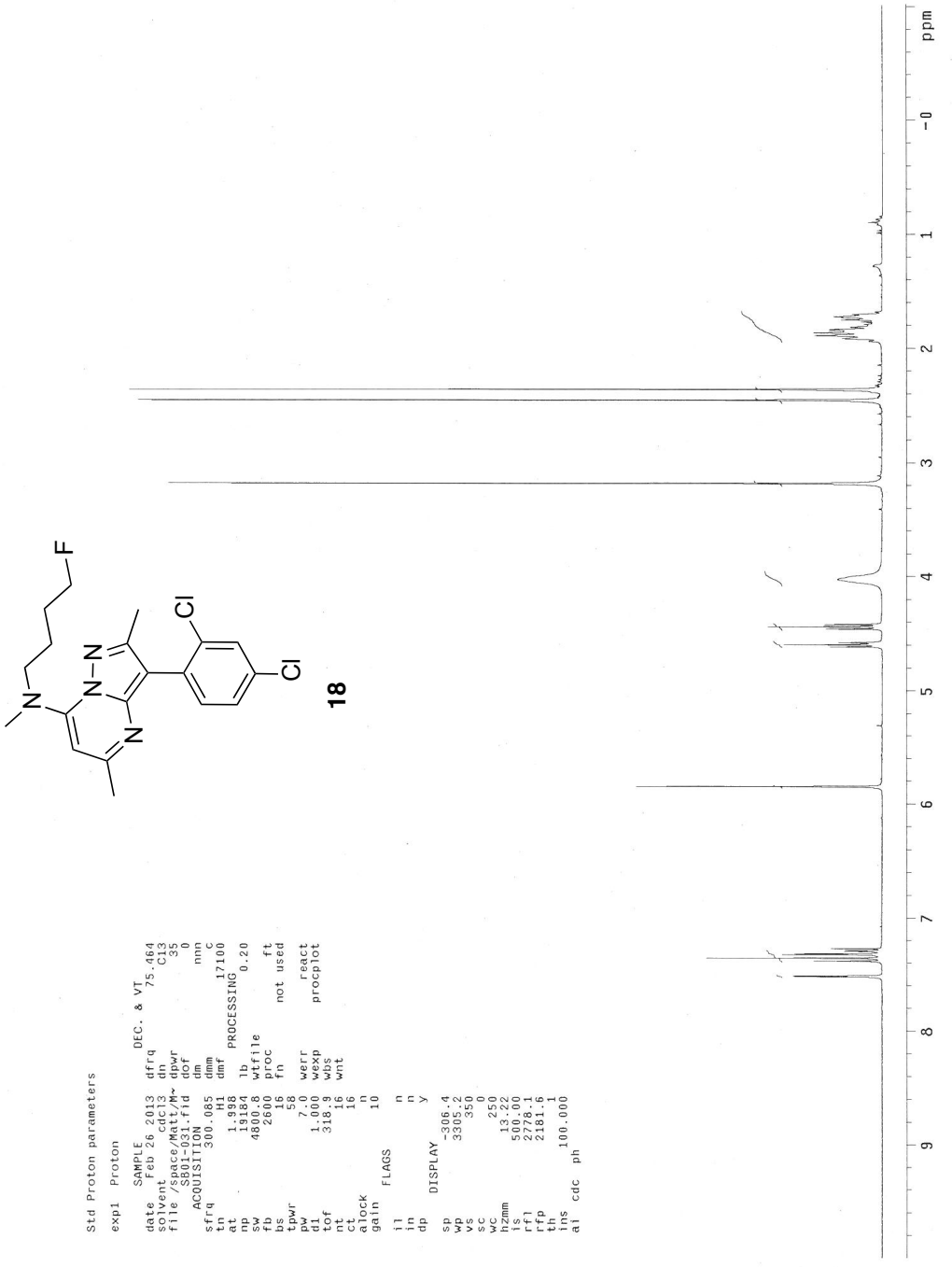
Spectrum 6. ^{1}H NMR Spectrum (300 MHz, $CDCl_3$) of Compound **16**



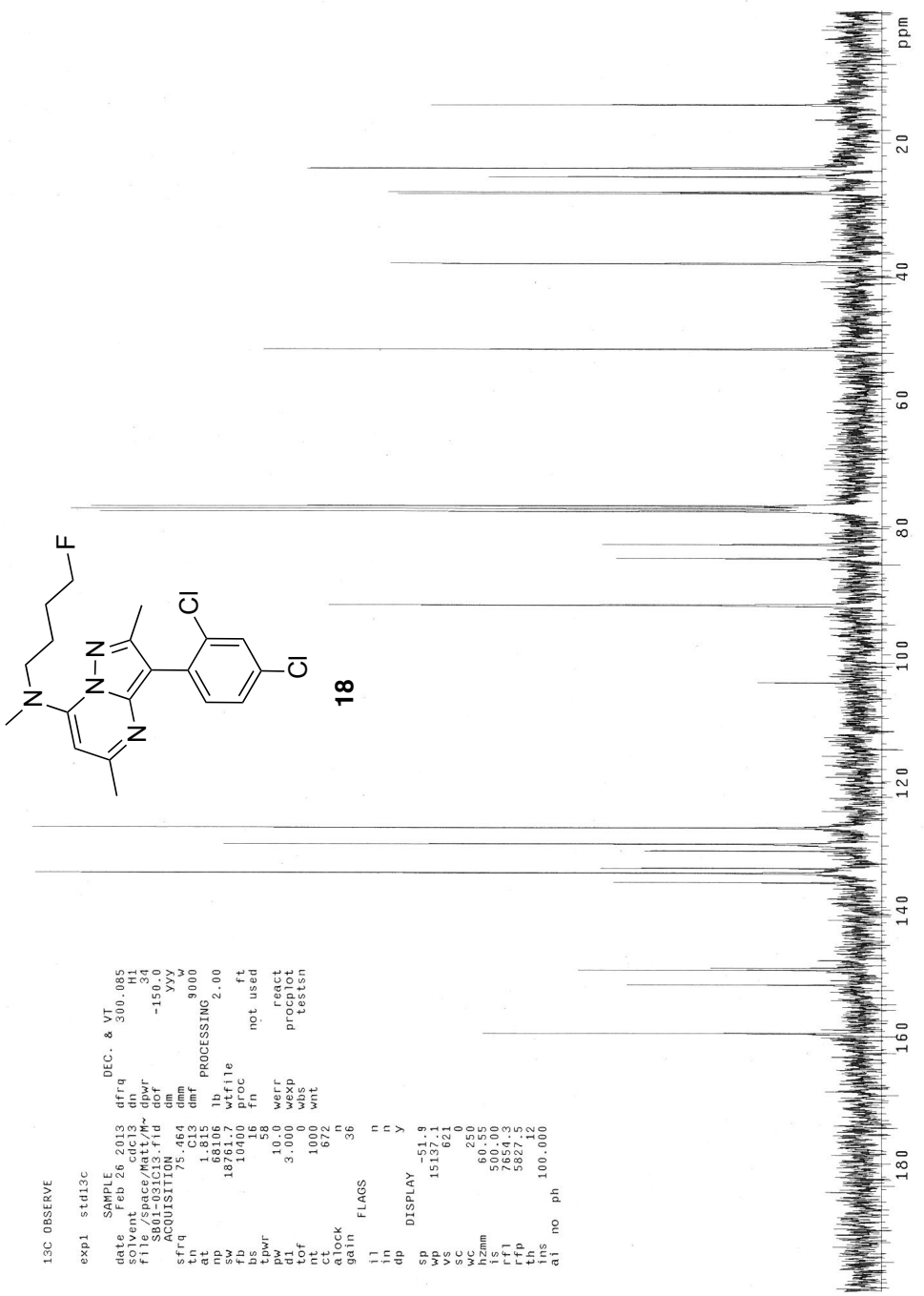
Spectrum 7. ^{13}C NMR Spectrum (75 MHz, $CDCl_3$) of Compound **16**

h

Spectrum 8. ^{1}H NMR Spectrum (300 MHz, $CDCl_3$) of Compound **17**



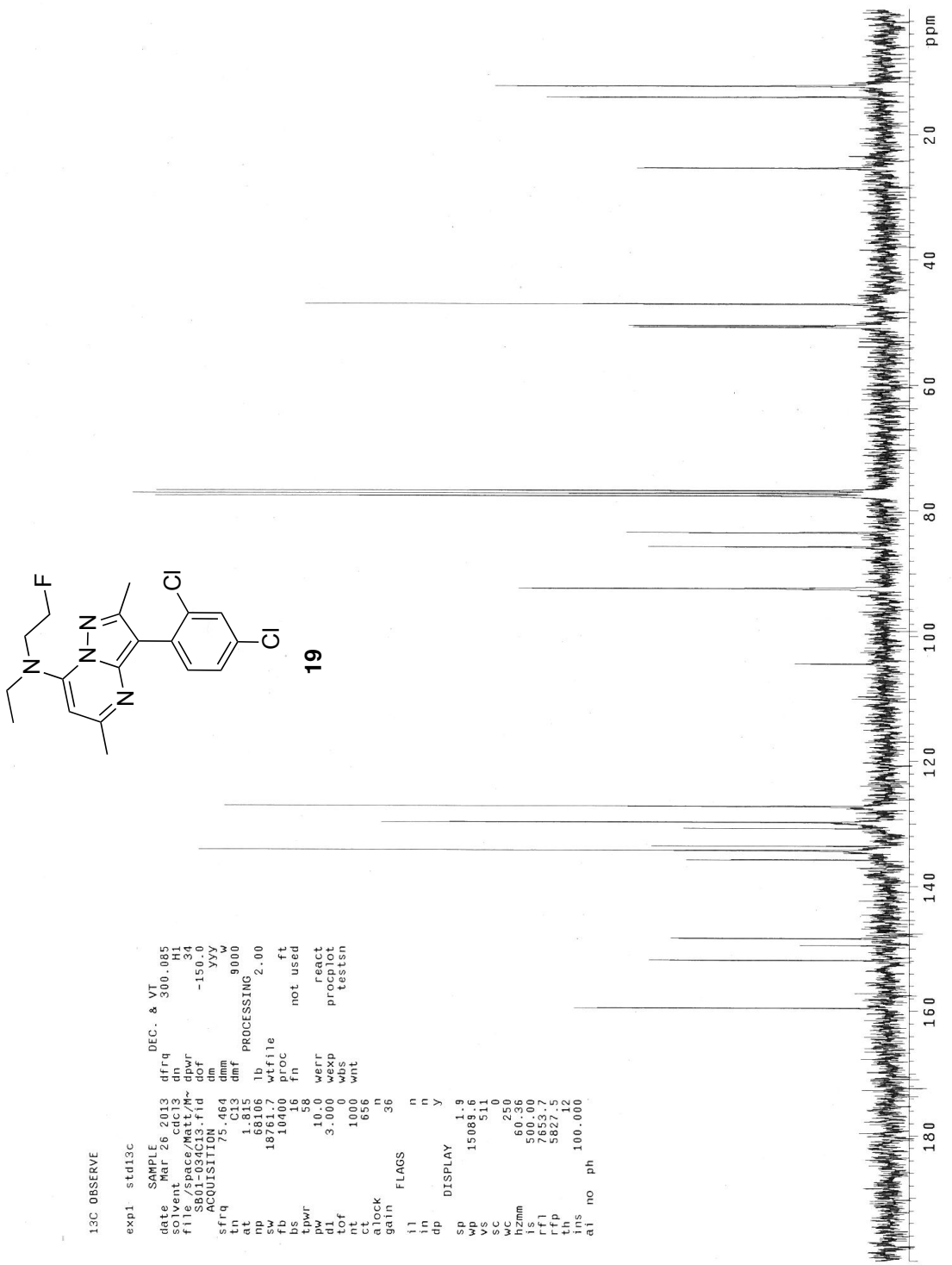
Spectrum 9. ^{1}H NMR Spectrum (300 MHz, $CDCl_3$) of Compound **18**



Spectrum 10. ^{13}C NMR Spectrum (75 MHz, $CDCl_3$) of Compound **18**

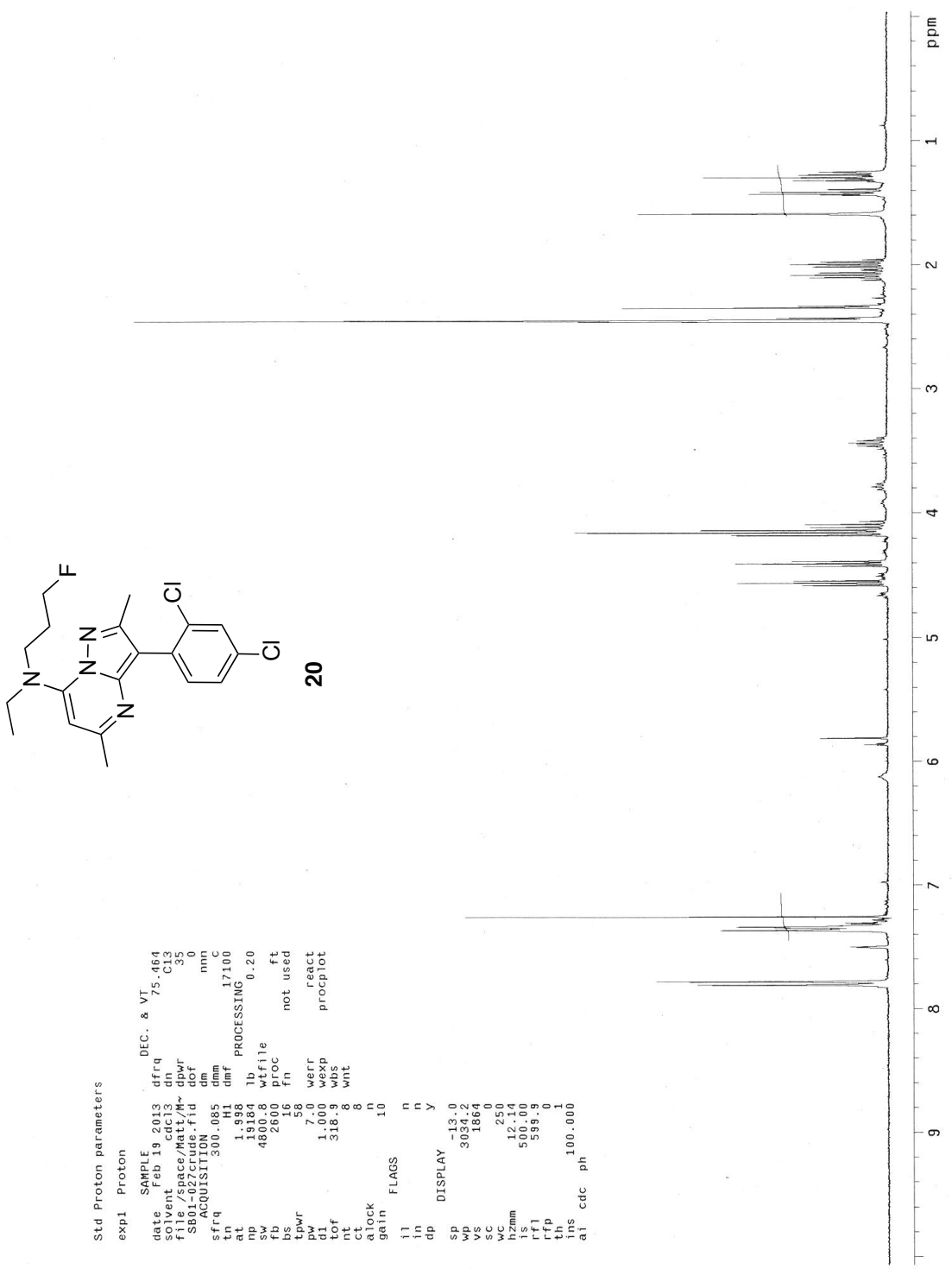
k

Spectrum 11. ^{1}H NMR Spectrum (300 MHz, $CDCl_3$) of Compound **19**



Spectrum 12. ^{13}C NMR Spectrum (75 MHz, $CDCl_3$) of Compound **19**

m



Spectrum 13. ^{1}H NMR Spectrum (300 MHz, $CDCl_3$) of Compound **20**

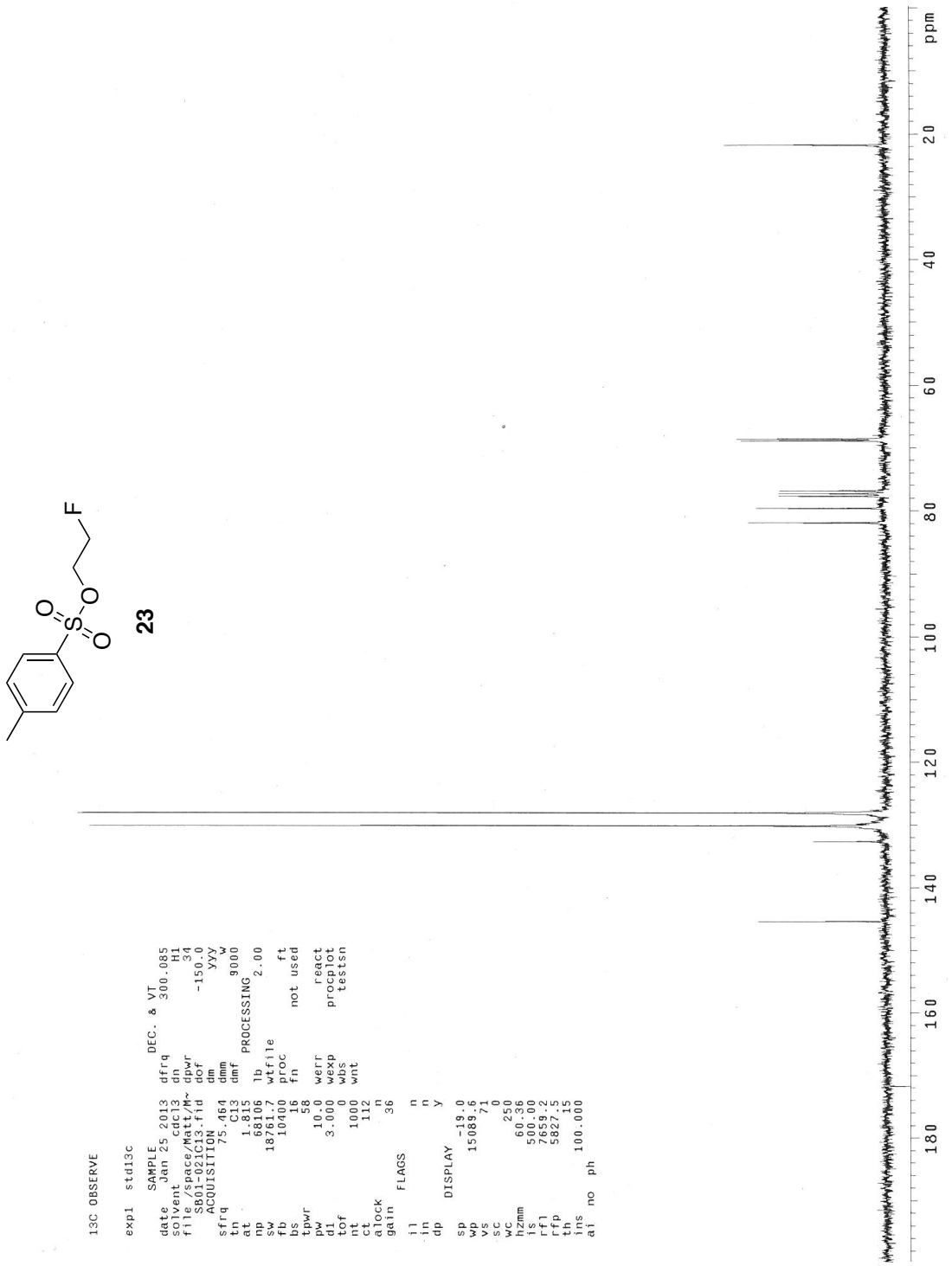
n

Spectrum 14. ^{1}H NMR Spectrum (300 MHz, $CDCl_3$) of Compound **21**

Spectrum 15. ^{1}H NMR Spectrum (300 MHz, $CDCl_3$) of Compound **22**

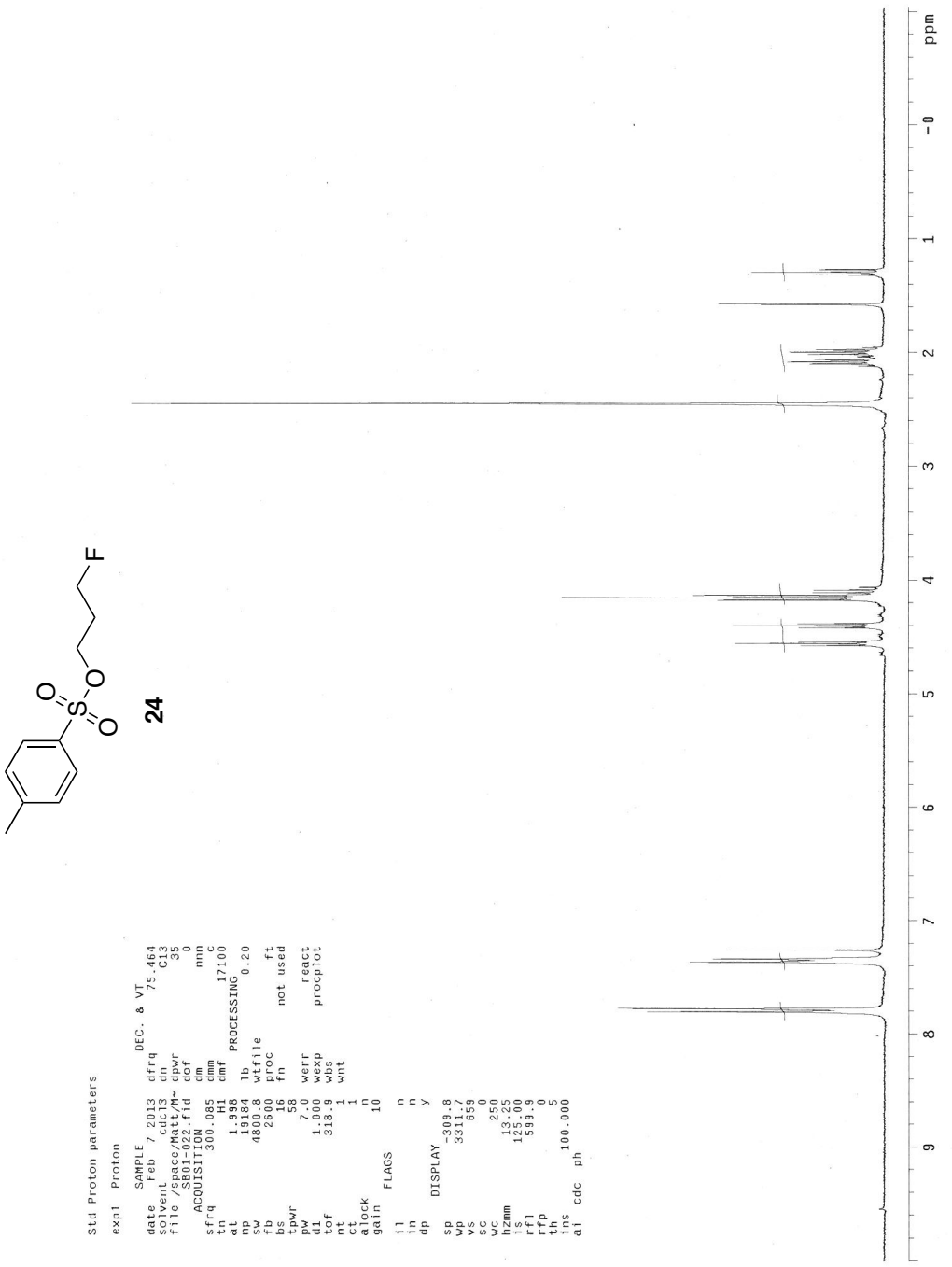
p

Spectrum 16. ^{1}H NMR Spectrum (300 MHz, $CDCl_3$) of Compound **23**



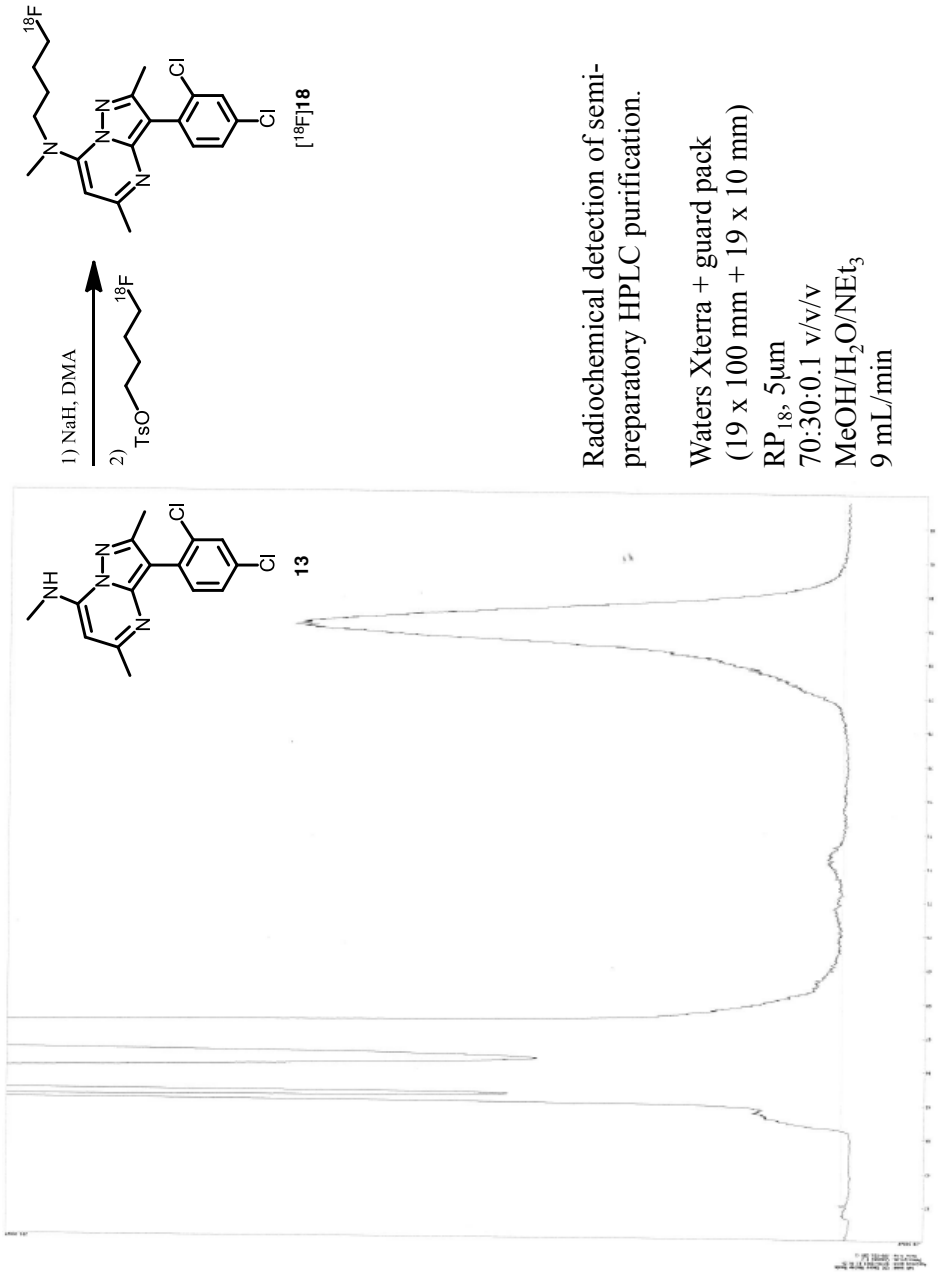
Spectrum 17. ^{13}C NMR Spectrum (75 MHZ, $CDCl_3$) of Compound **23**

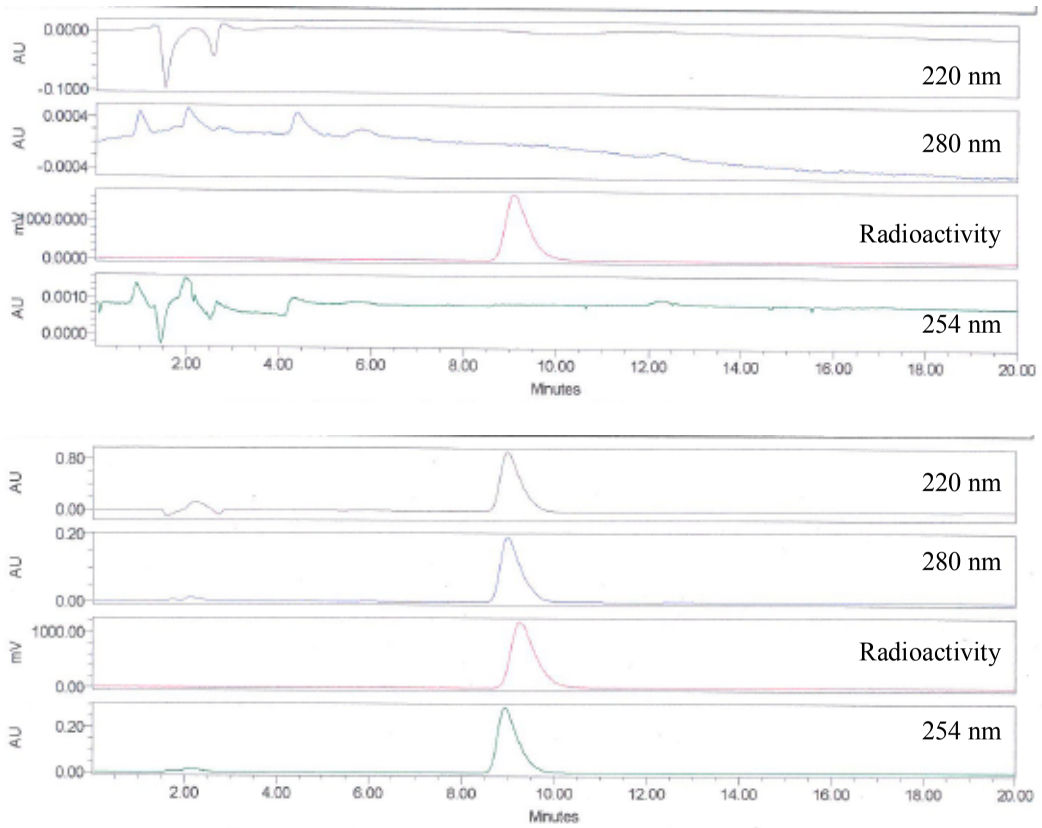
r

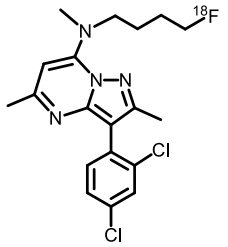


Spectrum 18. ^{1}H NMR Spectrum (300 MHz, $CDCl_3$) of Compound **24**

Appendix II. HPLC Data for Radiolabeling







[^{18}F]**18**

Coinject with cold standard.

Analytical HPLC (Waters NovaPak, 3.9 mm x 150 mm, 75:25:0.1 v/v/v MeOH/H_2O/NEt_3, 1 mL/min).