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GPR55 is a G-protein coupled receptor (GPCR) that was first discovered in 1990 and deorphanized in 2006. A potent agonist of GPR55 is the endogenous LPI and one antagonist is ML192. Due to the diseases associated with GPR55, a more potent antagonist or agonist is required that can deactivate or activate the receptor in order to further elucidate the role and function of GPR55 in the body. The focus of the research here in is the synthesis a variety of analogs of ML192 with the goal of finding a more potent antagonist than ML192. The synthesized analogs were subjected to biological assays and computational analysis. Four different novel analogs were synthesized that further probed some structure-activity relationships

.

SYNTHESIS OF POTENTIAL POTENT ANTAGONIST

OF G-PROTEIN COUPLED RECEPTORS

by

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A Thesis Submitted to
the Faculty of The Graduate School at
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Master of Science

Greensboro
2016

Approved by

_____
Committee Chair

DEDICATION

I dedicated this to my parents for their endless support and for standing by me through everything in my life. Your hugs, laughs, and jokes were the light that kept me going.

I love you always and forever from the bottom of my heart.

"Be kind to your parents; if one or both of them live to their old age in your lifetime, you shall not say to them any word of contempt nor repel them and you shall address them in kind words. (Holy Qur'an Surah 17, Ayah 23)"

APPROVAL PAGE

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Date of Final Oral Examination

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LIST OF ABBREVIATIONS

δ	chemical shift in parts per million downfield from tetramethylsilane
° C	degrees Celsius
μ	micro
DMSO	dimethyl sulfoxide
GPCR	g protein coupled receptor
ESI	electrospray ionization
EtOAc	ethyl acetate
Hz	hertz
L	liter
M^+	parent molecular ion
m/z	mass to charge ratio
MeOH	methanol
mL	milliliter
mmol	mmol
mol	mole
NMR	nuclear magnetic resonance
ppm	parts per million
TLC	thin layer chromatography
1H NMR	proton nuclear magnetic resonance
13C NMR	carbon-13 nuclear magnetic resonance

IC_{50}	50% inhibitory concentration
TMH	transmembrane helix
C10	cysteine at position 10
C260	cysteine at position 260
C3.25	cysteine at position 3.25
C168	cysteine at position 168
TMH3	transmembrane helix 3
TMH6	transmembrane helix 6
R3.50	arginine at position 3.50
Q6.30	glutamine at position 6.30
Q7.36	glutamine at position 7.36
S7.32	serine at position 7.31
K2.60	lysine at position 2.60
mg	milligram
BOC	tert-butyloxycarbonyl
$POCl_3$	phosphorus (iv) oxychloride
μM	micromolar
DMA	dimethylacetamide
br	broad
M	mole per liter
FTMS	fourier transform mass spectrometry

KOH	potassium hydroxide
DCM	dichloromethane

CHAPTER I

INTRODUCTION

G-Protein Coupled Receptors (GPCRs) are integral transmembrane proteins that are found all over the body such as the central nervous system, lungs, and bone.[1] GPCRs are one of the largest superfamily of proteins that consists of approximately 1000 types.[i] Due to their biological activity, 60% of pharmaceutical drugs target GPCRs.[1]

GPR55 is a GPCR that was first identified and cloned in 1990 and deorphanized in 2006.[2] GPR55 is composed of seven transmembrane helices (TMH, Figure 1). Some biological activities of GPR55 include inflammation, cancer, and pain.[3,4] Studies showed that GPR55 is up regulated in a variety of cancers such as ovarian, breast, and pancreatic cancer.[4] There is a direct correlation found between the progression of certain cancers and the amount of GPR55 activity.[4] This makes GPR55 a potential drug target for various diseases and treatments.

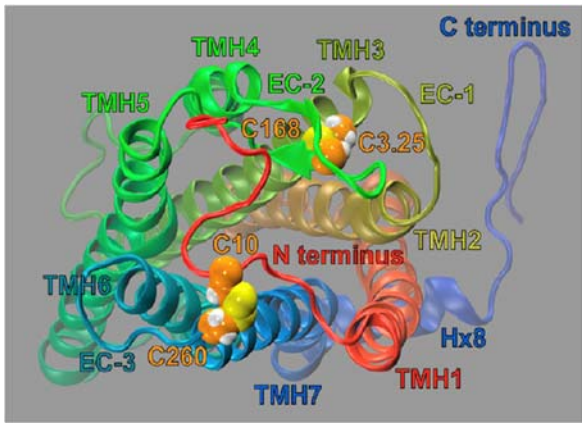
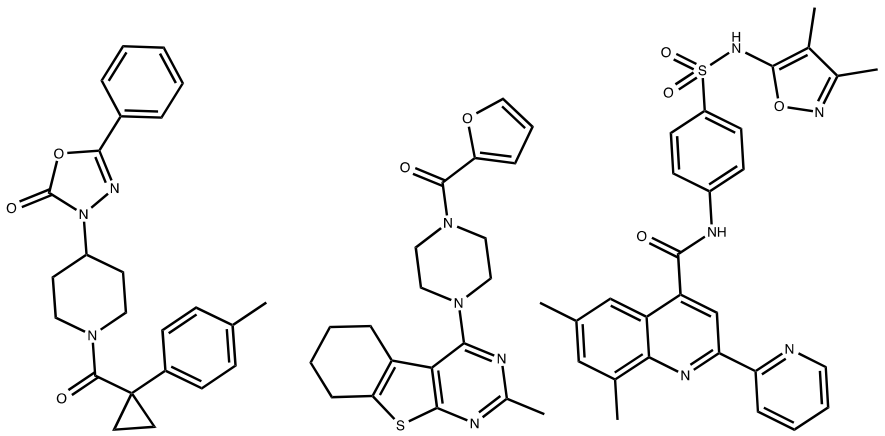


Figure 1. GPR55 Receptor, with the Disulfide Bridges Depicted. Reprinted with permission from *Biochemistry*. Copyright 2013 American Chemical Society.[3]

A study was completed on knockout mice in order to determine the biological effects of GPR55.[4] One of the effects in mice was long-term depression when higher levels of GPR55 was observed.[4] Another study showed that GPR55 disrupts the activation of decidual cells, which leads to fetoplacental development.[i5] GPR55 is expressed in the following types of cells: bone marrow, endothelial cells, and mast cells.[6,4] The role that GPR55 has on psychological and physiological effects is not known but it is associated with many diseases, tissues, cells, and organs.

Due to the impressive biological activity of GPR55, a screening of approximately 300,000 compounds was completed by the Sanford-Burnham Medical Research Institute.[3] The compounds were tested by using β-arrestin assays to identify antagonists of GPR55.[3] This led to the identification of three potent antagonist scaffolds of GPR55: ML191, ML192, and ML193 with IC_{50} values of 1.08 (±0.03) μM, 0.70 (±0.05) μM,

and 0.22 (±0.03) μM, respectively (Figure 2).[3] Docking studies were then completed by Dr. Reggio's research group on the three antagonist scaffolds.[3]



	CID 23612552 ML 191	CID 1434953 ML 192	CID12161822 ML 193
IC_{50}	1.08 ± 0.03 μM	0.70 ± 0.05 μM	0.22 ± 0.03 μM

Figure 2. Structures of GPR 55 Antagonists.[3]

The docking studies used a homology model of GPR55 in the active and inactive states, which are based off of the β-adrenergic receptor. There were two disulfide bridges on GPR55 which are between residues C10 and C260 and residues C3.25, and C168 (Figure 1).[iii] The other two key structural features on GPR55 were the ionic lock and "toggle switch" residues. Those residues had vital roles on the receptor when an antagonist or agonist binds. The "toggle switch" was on TMH3 and TMH6, consisting of residues M3.36 and M6.48.[iii] The ionic lock is a hydrogen bond that occurs between R3.50 and Q6.30.[iii] When the receptor is activated by an agonist, the toggle switch

residues are rotated away from each other due to the straightening of TMH6.[iii] This caused the breaking of the ionic lock and allowed for the G-protein to bind.

The docking studies on antagonist ML192 showed the toggle switch residue M3.36 and M6.48 had interactions that will prevent the straightening of TMH6 and, therefore, ML192 acted as an antagonist (Figure 3).[iii] There were three hydrogen bonding interactions that are shown in yellow in Figure 3. The hydrogen bonding interaction between ML192 and K2.60 accounts for the majority of the interaction.[3] The hydrogen bonding to K2.60 used the most electronegative region of ML192 according to the electrostatic potential maps (Figure 4).[3] The other hydrogen bonding interactions that occurred were between Q7.36 and both K2.60 and ML192.[3] All of the docking studies completed on ML191, ML192, and ML193 showed similar interactions due to the similarities in the structure and the electrostatic potential maps (Figure 4).[3]

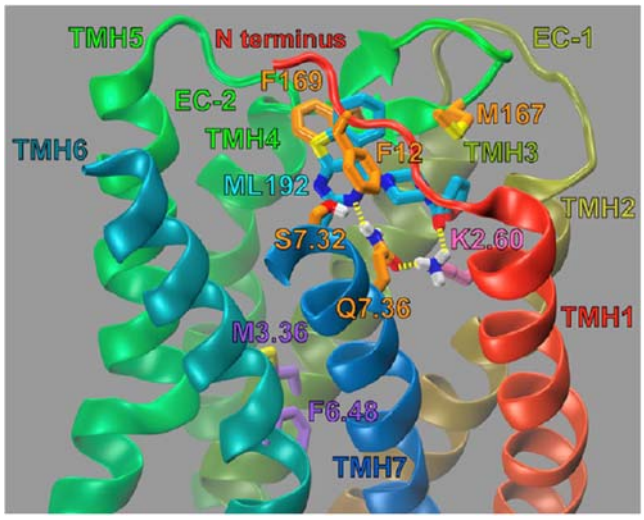


Figure 3. Antagonist, ML192, Docked into GPR55 Receptor, Toggle Switch Residues are Shown in Purple. Reprinted with permission from *Biochemistry*. Copyright 2013 American Chemical Society.[3]

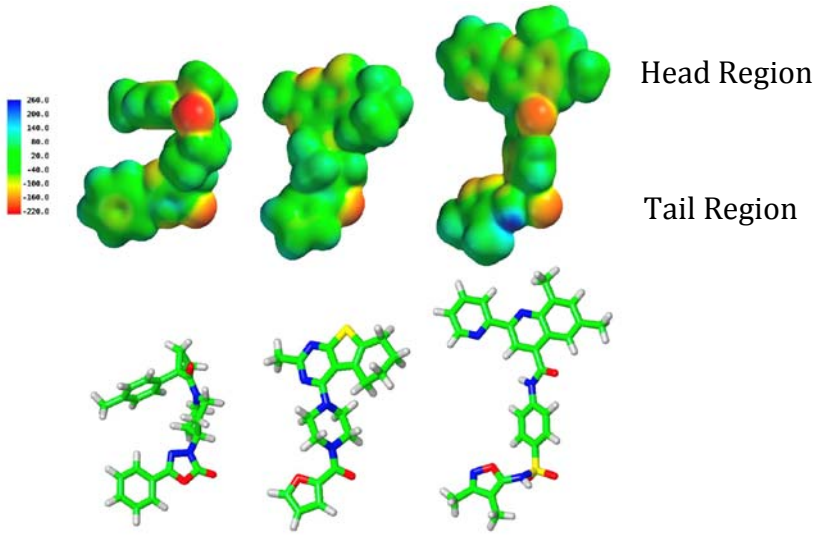


Figure 4. Electrostatic Potential Map of ML191, ML192, and ML193 (from left to right). Reprinted with permission from *Biochemistry*. Copyright 2013 American Chemical Society.[3]

As Shown in Figure 4, these compounds have three similar structural features: a head region, a linear portion, and an aromatic, typically heteroaromatic portion. The head region interacts with the extracellular loops of the receptor.[3]The linear region contains the most electronegative portion which contributed to the majority of the docking interaction.[3] The aromatic or heterocyclic portion, or tail region, interacts with the bottom of the binding pocket of GPR55.[3] All of these structural features are seen in Figure 4.

Compound ML192 was the focus scaffold of the research herein. Analogs were typically synthesized on a 10 mg scale and submitted for biological testing by Dr. Abood at Temple University. The analogs of ML192 will further elucidate interactions between the receptor and the analog. The goal of the research was to find analogs that are more potent than the parent compound (ML192).

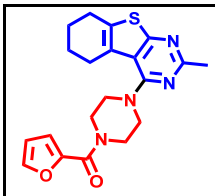
CHAPTER II

SYNTHESIS OF GPR55 ANTAGONISTS

2.1 Aims of Research

ML192 was the parent compound (**1**) that diversification was focused on, due to the fact that ML193 has many commercial analogs available, and preliminary results of scaffold ML191 showed no promising potent antagonist of GPR55 when diversification of analogs was completed (Figure 1).[7] Therefore, the synthesized analogs focused on diversification of ML192 (**1**). The analogs synthesized will focus on diversification of the head region (Figure 4; Table 1). The goal was to synthesize a more potent analog and decipher major or minor interactions between the analog and receptor. For all the analogs synthesized, the furoyl piperazine remained constant due to the fact that when the furan was replaced with a thiophene or 1-methyl-1H-pyrrole the IC_{50} ranges increased (Table 2).

Table 1. Parent Compound and Analogs of the Compound



Analog 1
ML192
Parent Compound

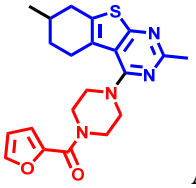
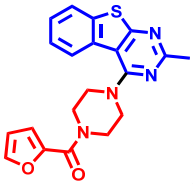
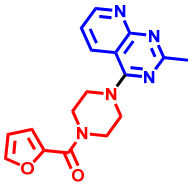
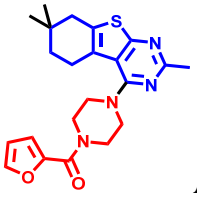
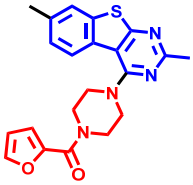
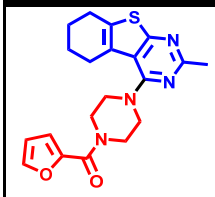
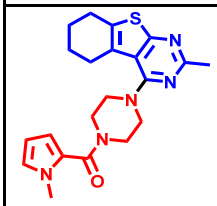
Aim 1	Aim 2	Aim 3
 **Analog 2**	 **Analog 4**	 **Analog 6**
 **Analog 3**	 **Analog 5**	

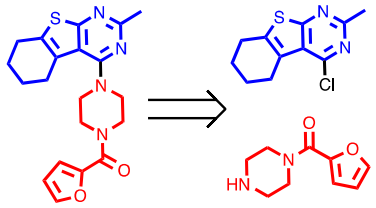
Table 2. Diversification of the Piperazine

STRUCTURE	IC_{50} Value
	3.7-10 μM
	13-14 μM

The aims of this thesis are as to synthesize a small library of compounds to obtain more structure-activity relationships for ML192. Analogs **2-3** were designed to see the

effect of methyl groups at position 7 (Aim 1). Analogs **4-5** showed the effect of an aromatic group (Aim 2). Analog **6** focused on the effect of a bicycle instead of a tricycle (Aim 3). The synthesis and resulting activities will be described in the subsequent sections.

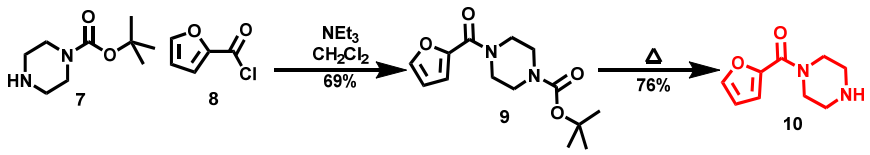
Synthesis of the analogs was accomplished through a coupling of furoyl piperazine (in red) and chloropyrimidine (in blue, Scheme 1). The coupling was performed through a nucleophilic aromatic substitution reaction. The furoyl piperazine chain remained a constant for all five analogs synthesized.



Scheme 1. The Nucleophilic Aromatic Substitution Reaction Completed to Couple the Two Fragments.

2.2 Synthesis of Furoyl Piperazine

The furoyl piperazine was synthesized in two steps from commercially available starting materials (Scheme 2). The 1-Boc-piperazine (**7**) was reacted with 2-furoyl chloride (**8**) and yielded a 73% yield of a furoyl boc protected piperazine (**9**).[8] The deprotection of the Boc group was accomplished through thermolysis, which yielded a 76% of furoyl piperazine chain **10**.[9] Furoyl piperazine **10** was reacted in a nucleophilic aromatic substitution reaction (Scheme 1).

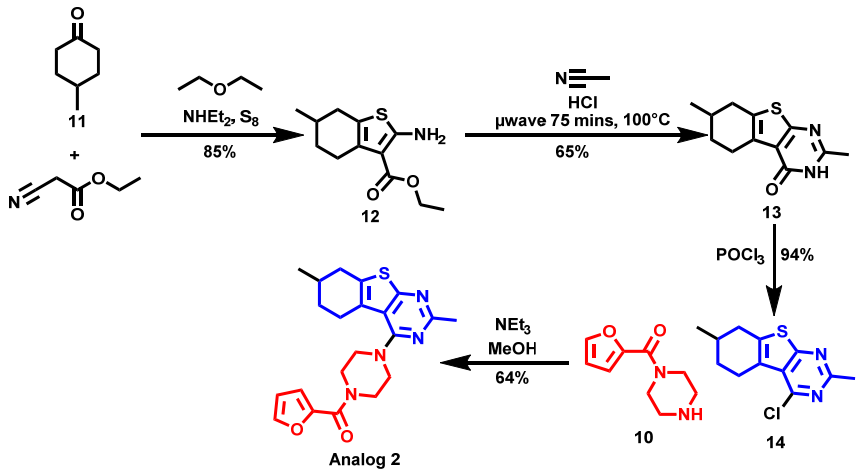


Scheme 2. Synthesis of the Furoyl Piperazine Required for all Analogs.

2.3 Aim 1- Synthesis of Methyl Analogs

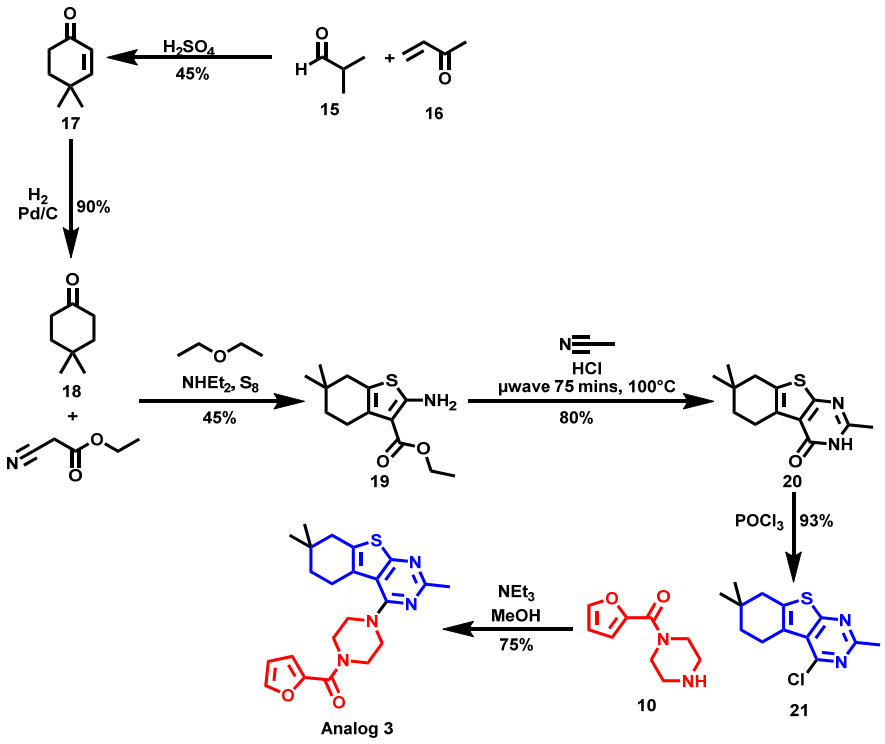
Aim 1 was the synthesis of analogs **2** and **3**. The analogs were synthesized through a nucleophilic aromatic substitution reaction in which furoyl piperazine (**10**) was reacted with a chloropyrimidine. The chloropyrimidine core was synthesized in three reactions from commercially available starting material.

For the synthesis of analog **2**, 4-methyl-cyclohexanone (**11**) was reacted in a Gewald reaction resulting in amino ester **12** that underwent a cyclization using hydrochloric acid and acetonitrile yielding pyrimidinone **13** (Scheme 3).[10, 11] The last reaction was the conversion of the pyrimidinone to the chloropyrimidine (**14**).[12] The chloropyrimidine (**14**) and furoyl piperazine (**10**) was reacted in a nucleophilic aromatic substitution reaction resulting in analog **2**.[12]



Scheme 3. Synthesis of the Chloropyrimidine Required for Analogs 2.

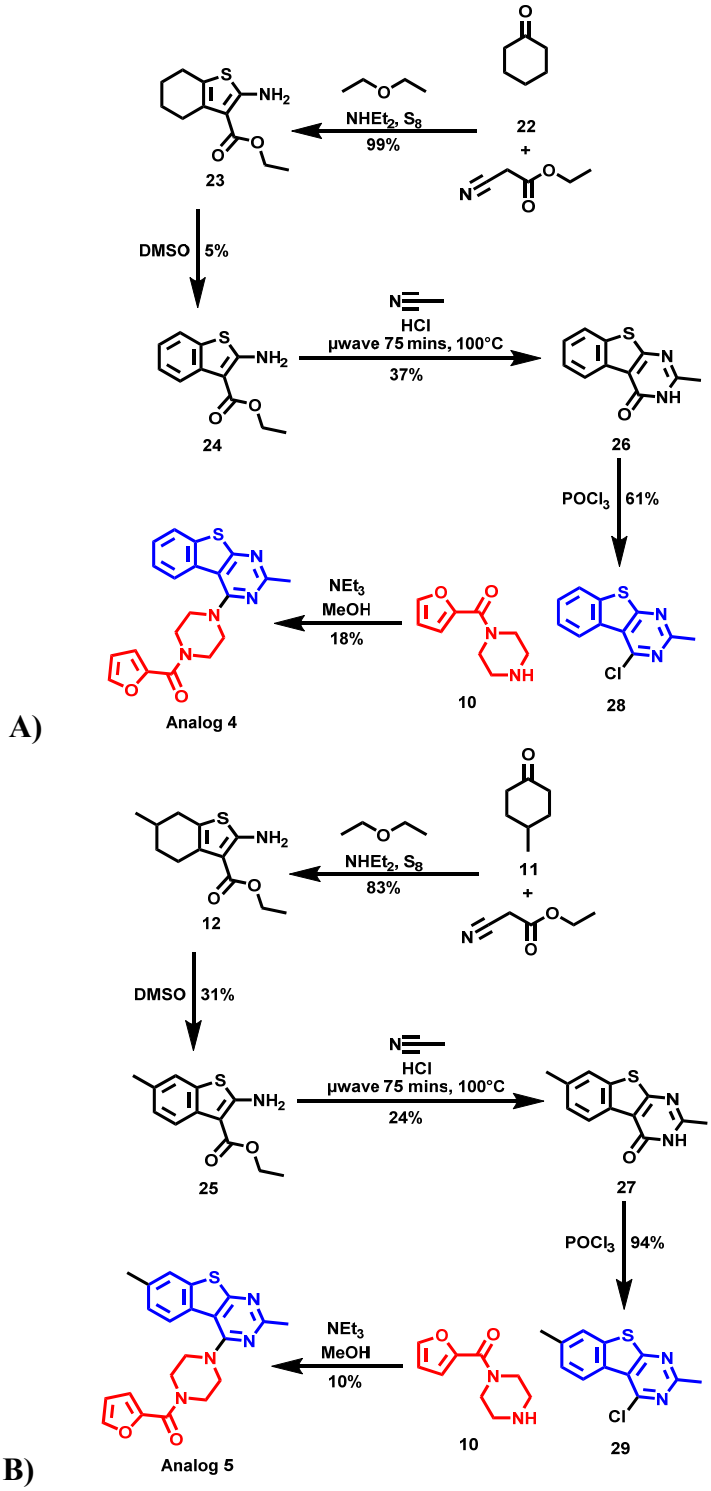
Unlike analog **2**, the ketone required for analog **3** was synthesized through an acid catalyzed Robinson annulation reaction of isobutyraldehyde (**15**) and methyl-vinyl ketone (**16**) yielding 4,4-dimethyl-cyclohex-2-enone (**17**; Scheme 4).[13, 14] The alkene was then hydrogenated using palladium on carbon reaction yielding 4,4,-dimethyl cyclohexanone (**18**). The ketone (**18**) then underwent a Gewald reaction yielding amino ester **19** which was then cyclized to a pyrimidinone (**20**). The pyrimidinone (**20**) was then reacted with phosphorus (IV) oxychloride ($POCl_3$) that formed the chloropyrimidine (**21**), required for the nucleophilic aromatic substitution reaction.



Scheme 4. Synthesis of the Chloropyrimidine Required for Analogs 3.

2.4 Aim 2- Synthesis of Aromatic Analogs

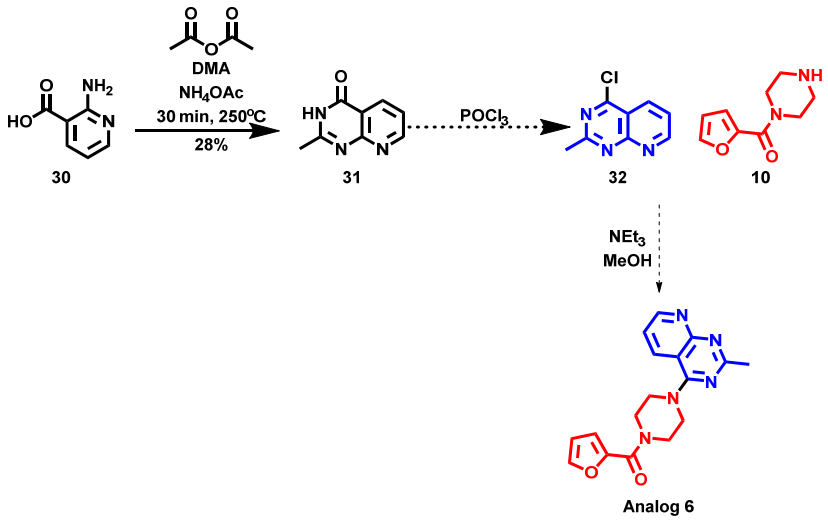
Both analogs **4** and **5** were synthesized in a series of four reactions (Scheme 5). The first reaction is the Gewald reaction that used ketone **22** or **11** and formed amino ester **23** or **12**. Those were then oxidized using DMSO which yielded aromatic amino ester **24** or **25**, which were then cyclized to pyrimidinones **26** or **27**.[15] The carbonyl oxygen of the pyrimidinone was converted to a chloride and yielded an aromatic chloropyrimidine, **28** or **29**. The aromatic chloropyrimidine (**28** or **29**) was then reacted in a nucleophilic aromatic substitution reaction which yielded analogs **4** or **5**.



Scheme 5. A) Synthesis of Analog 4 B) Synthesis of Analog 5.

2.5 Aim 3- Synthesis of Bicycle Analog

The final analog (**6**) requires the synthesis of a bicycle instead of the tricycle that is seen in analogs **2**, **3**, **4**, and **5**. The bicycle will be synthesized in a two-step reaction (Scheme 6). The first reaction reacted 2-aminopyridine-3-carboxylic acid (**30**) with dimethylacetamide (DMA), ammonium acetate, and acetic anhydride to generate pyrimidinone **31**.[16] The pyrimidinone will then be chlorinated using $POCl_3$, yielding a bicycle (**32**) that will undergo a nucleophilic aromatic substitution reaction. The chlorination and nucleophilic aromatic substitution reactions are still being optimized.

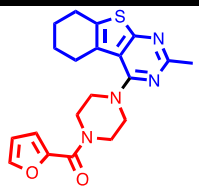
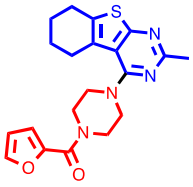
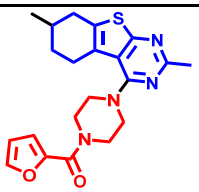
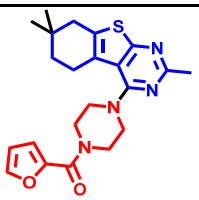
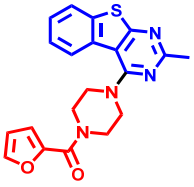
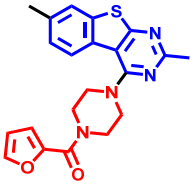


Scheme 6. Synthesis of the Aromatic Bicycle Core Required for Analog 6.

2.6 Biological Activity Results

There were a total of five compounds, ML192 and analogs **2**-**5**, that were synthesized and submitted for biological activity. The results that were obtained from Dr. Abood's group are seen in Table 2. The results showed that analog **3** has the most promising results with the lowest IC_{50} value range of 0.16-1.4 μM. This is an indication that having a dimethyl at position 7 is preferred, over no methyl (analog **1**) or one methyl group (analog **2**; Figure 5). The IC_{50} value of analog **2** represents a racemic mixture of the chiral center at position 7 (Figure 5).

Table 3. Biological Activity for Synthesized Analogs

PARENT COMPOUND ACTIVITY FROM LITERATURE[3]		
		0.70 ± 0.05 µM
ANALOG	**STRUCTURE**	**IC_{50}**
1		7.84 µM
2		0.5-7.1 µM
3		0.16-1.4 µM
4		**Sent for testing awaiting results**
5		**Sent for testing awaiting results**

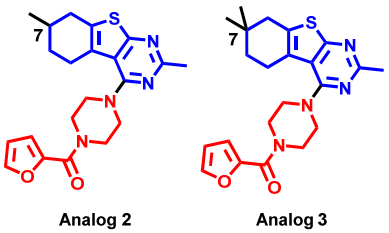


Figure 5. Position7 on Analogs 2 and 3.

2.7 Structure Activity Relationships Study

From the data obtained, and diversification that was completed on ML192, there are four major changes that were seen (Figure 6). Three of the changes will focus on the diversification on the "head" region and the last will be on the "tail" region of the ML192. Prior analogs showed the importance of the furan over a thiophene or pyrrole ring. These were the only changes that were completed on the "tail" portion of the analog (Figure 4, Table 2).

There are a total of four different analogs that focused on diversification of the cyclohexene. These modifications will be used to determine the effect of a methyl group (analogs **2** and **3**) and the effect of aromaticity (Figure 6, analogs **4** and **5**).

For the addition of a methyl group at position 7, a dimethyl is preferred over a single methyl for the following reasons (Figure 5). The compound with a methyl group (analog **2**) has a chiral center and pharmaceutical companies prefer the synthesis of achiral compounds or a synthesis that will result with a single enantiomer. Due to the fact that the dimethyl analog (analog **3**) has a similar, if not improved, activity compared to the monomethyl compound, a dimethyl group is preferred to remove the complexity of

the chirality. The addition of the methyl group showed that there are van der Waals interactions that occur between the receptor and the analog, which will be confirmed through subsequent computational studies.

The second change to the cyclohexene will show the effect of aromaticity for the tricyclic core (Figure 6). The cyclohexene ring of ML192 is transformed to a benzene ring for analogs **4** and **5**. Analog **5** has a methyl group it will be interesting to compare the results of assay to analogs **2** and **3**. The aromatic ring will show if there are any π-stacking or cation π-interactions that occur between the analog and GPR55.

When comparing the activities of analogs **2**, **3**, **4**, and **5**, three observations can be made. The first is that alkylation of the 7-position is desirable as shown by analogs **2** and **3**. The second will be whether or not cyclohexene is preferred over a benzene ring. The third change will determine the importance of the bicycle and the cyclohexene ring. If the activity of analog **6** was to decrease compared to the parent compound, then the cyclohexene ring is preferred. This would mean a tricycle is preferred over a bicycle (Figure 6).

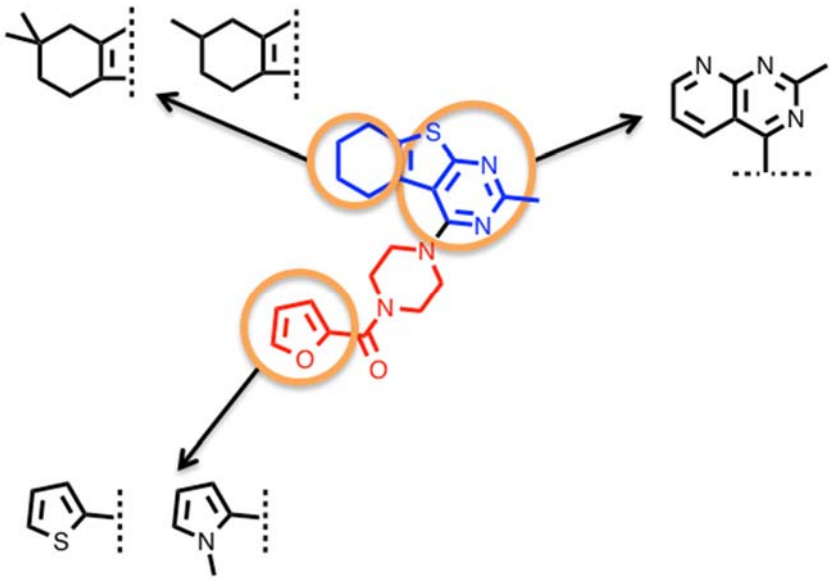


Figure 6. Summary of Structure-Activity Relationship on ML-192.

CHAPTER III

EXPERIMENTAL AND CHARACTERIZATION

3.1 General Information

All the reactions performed are under anhydrous conditions, which were performed using dry solvents in oven dried glassware under a nitrogen gas atmosphere. All the reagents and solvents were obtained commercially unless otherwise stated. Chromatographic purification was performed using silca gel (60 Å, 32-63 μm) unless otherwise stated. NMR spectra were recorded using JOEL ECA spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C) or JOEL ECS spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C). All NMRs were run at 25 °C unless otherwise stated. Coupling constants, *J*, are reported in hertz (Hz) and multiplicities are listed as singlet (s), doublet (d), triplet (t), doublet of doublet (dd), multiplet (m), etc. High resolution mass spectra were acquired on a ThermoFisher Scientific LTQ Orbitrap XL MS system.

3.2 Synthesis and Characterization of Amino Esters

All the Gewald reactions were run under the same conditions using a variety of different cyclohexanone derivatives (**11**, **18**, and **22**).

Synthesis of ethyl 2-amino-4, 5,6,7-tetrahydrobenzo [*b*] thiophene-3-carboxylate (synthesis of amino ester 24).[10,11]

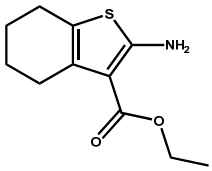
To an oven dry 50 mL round bottom flask were added ethylcyanoacetate (3.77 mL, 30.5 mmol), sulfur (0.978 g, 30.5 mmol), cyclohexanone (**22**) (3.29 mL, 30.5 mmol), diethyl ether (6.10 mL, 5.0 M), and diethyl amine (0.30 mL, 30.5 mmol). The flask was stirred under inert atmosphere at room temperature for 12 hours. The addition of the diethylamine turned the reaction red. The desired product (amino ester **23**) precipitated out of solution and was obtained through a filtration with cold water. There was a 62% yield of yellow solid (4.66 g, 20.7 mmol), which was confirmed by ^{1}H NMR as compared with literature data.[17]

Synthesis of ethyl 2-amino-6-methyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (synthesis of amino ester 12). [10,11]

The synthesis of amino ester **12**, used ketone **11** (2.42 mL, 26.8 mmol), under the same conditions mentioned above (synthesis of amino ester **23**). Amino ester **12** was synthesized in 81% yield, of an orange solid (5.27 g, 22.0 mmol), which was confirmed by ^{1}H NMR.[18]

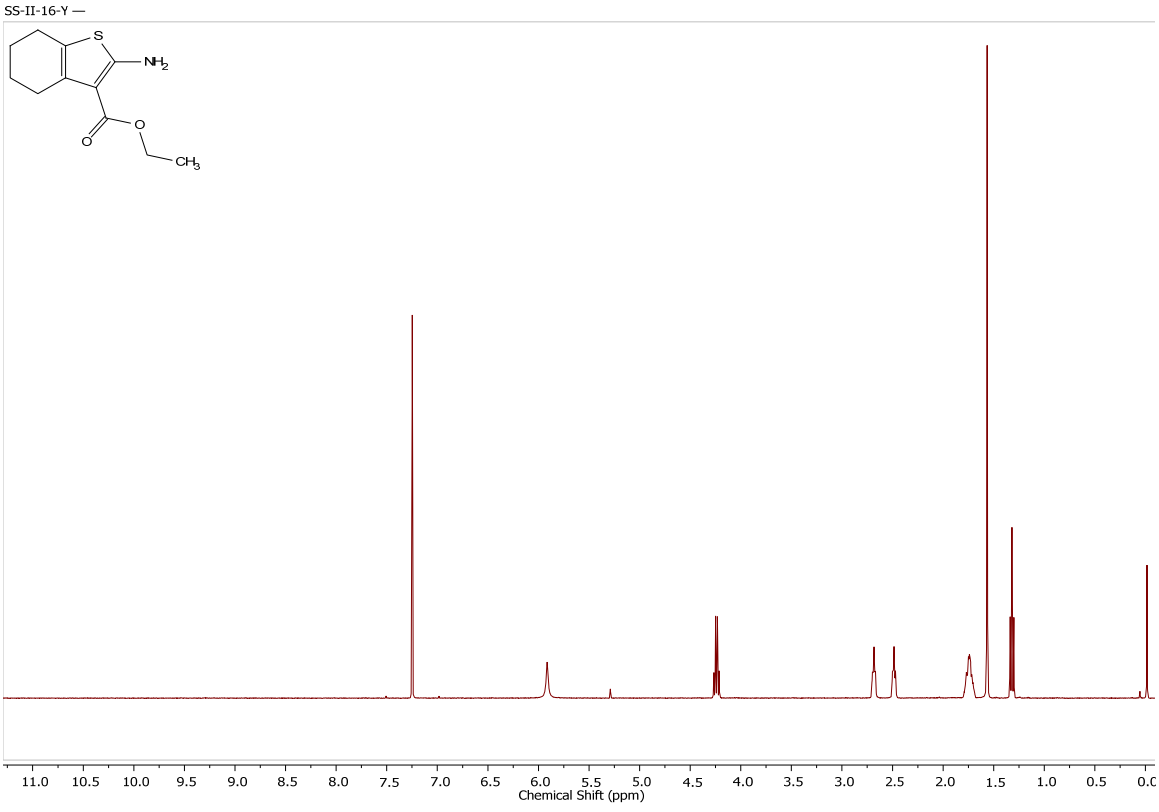
Synthesis of ethyl 2-amino-6,6-dimethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (synthesis of amino ester 19). [10,11]

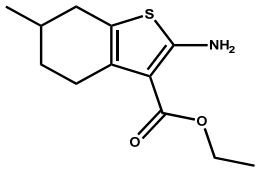
The synthesis of amino ester **19** was completed using ketone **18** (0.99 g, 7.89 mmol). Using the same reaction condition mentioned above (synthesis of amino ester **23**). Amino ester **19** was synthesized in 45% yield, of a red solid (0.89 g, 0.352 mmol), which was confirmed by ^{1}H NMR.



23

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.91 (br. s., 2H), 4.24 (q, *J* = 6.9 Hz, 2H), 2.72 - 2.64 (m, 2H), 2.48 (t, *J* = 6.0 Hz, 2H), 1.80 - 1.68 (m, 4H), 1.32 (t, *J* = 7.1 Hz, 3H) ppm.



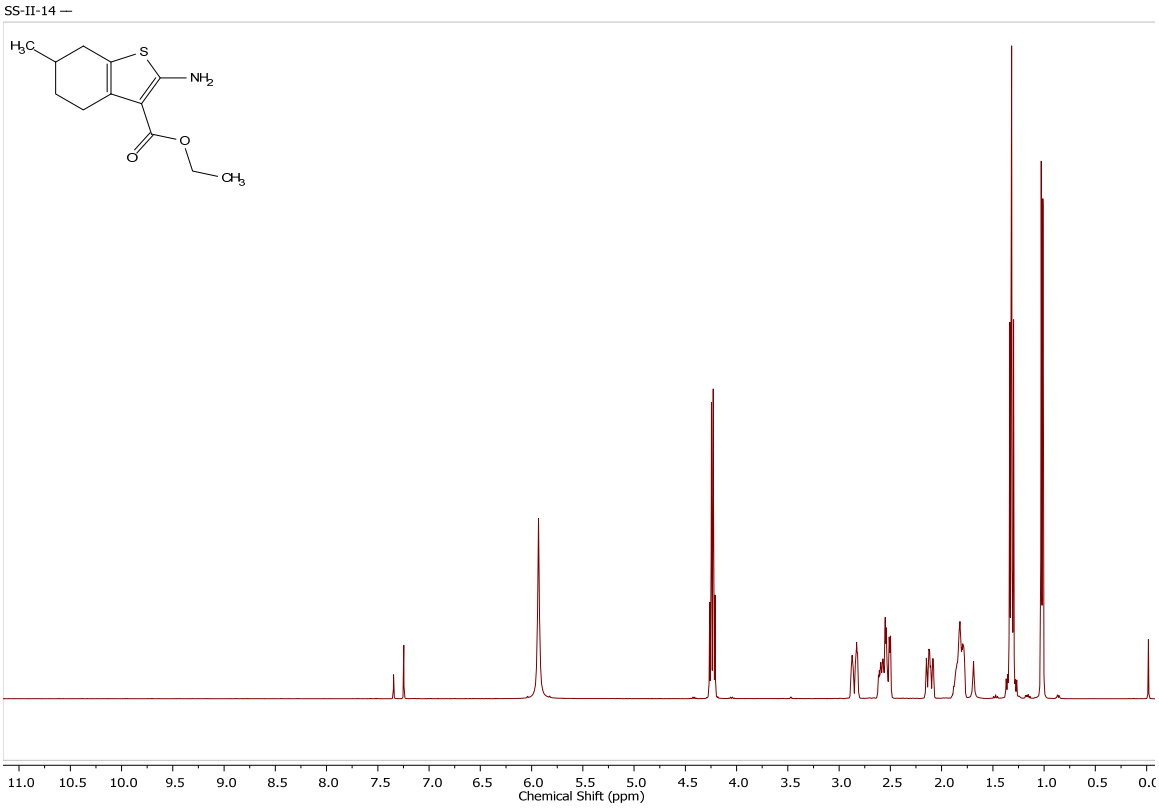


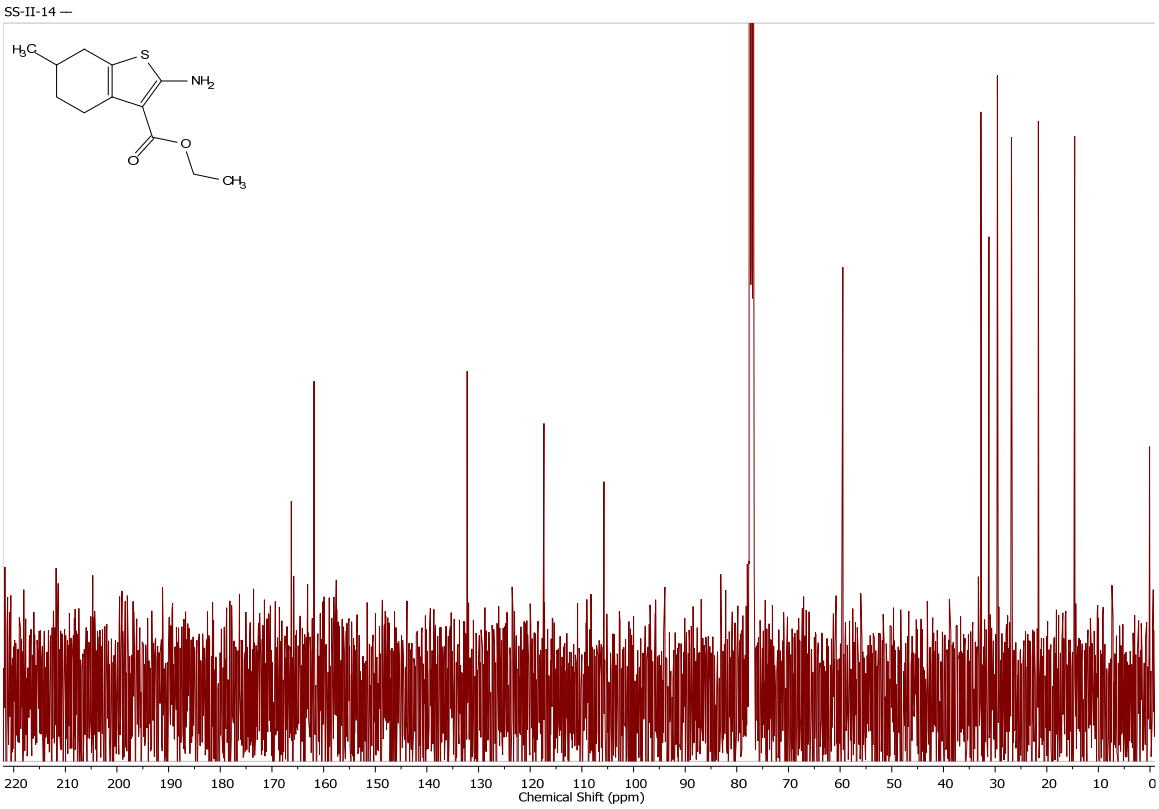
12

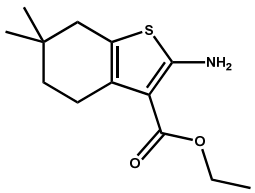
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.93 (br. s, 2H), 4.24 (q, J = 6.9 Hz, 2H), 2.85 (td, J_t = 2.9, J_d = 17.6 Hz, 1 H), 2.46 - 2.64 (m, 2H), 2.01 - 2.22 (m, 1H), 1.74 - 1.96 (m, 2H), 1.23 - 1.42 (m, 4H), 1.02 (d, J = 6.4 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 161.9, 132.2, 117.3, 105.7, 59.5, 32.7, 31.2, 29.6, 26.9, 21.6, 14.6 ppm.

FTMS ESI: $C_{12}H_{18}NO_2S$ $[M+H]^+$, Calculated: 240.1058 g/mol, Found: 240.1048 g/mol.





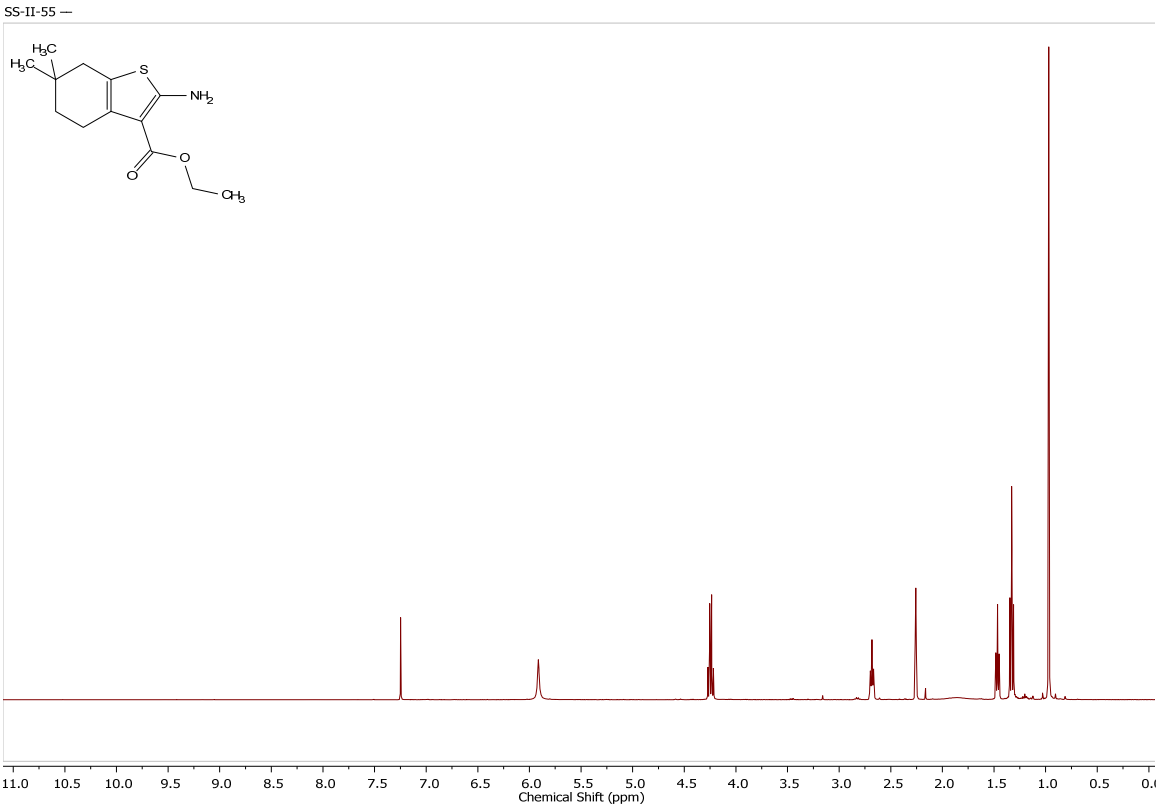


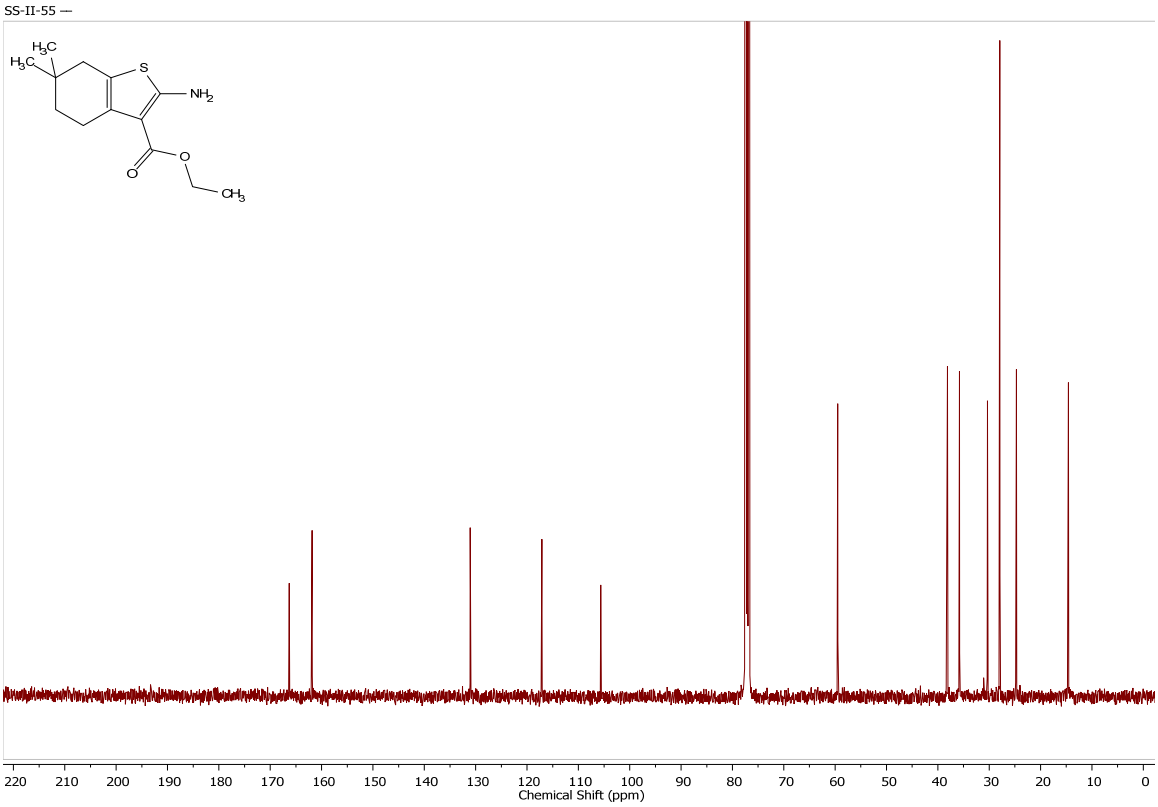
19

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.91 (br. s, 2H), 4.24 (q, *J* = 6.9 Hz, 2H), 2.68 (tt, *J* = 6.4, 1.8 Hz, 2H), 2.23 - 2.28 (m, 2H), 1.47 (t, *J* = 6.4 Hz, 2H), 1.33 (t, *J* = 7.1 Hz, 3H), 0.97 (s, 6H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 161.9, 131.1, 117.1, 105.6, 59.5, 38.1, 35.8, 30.3, 27.9, 24.7, 14.6 ppm.

FTMS ESI: $C_{13}H_{20}NO_2S$ $[M+H]^+$, Calculated: 254.1215 g/mol Found: 254.1202 g/mol.

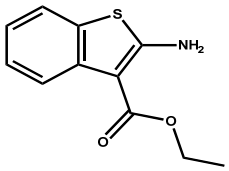

SS-II-55 —
H3C
H3C
S
NH2
O
O
CH3
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Chemical Shift (ppm)



3.3 Synthesis and Characterization of Aromatic Amino Ester Derivatives [15]

Synthesis of ethyl 2-aminobenzo[*b*]thiophene-3-carboxylate (aromatic amino ester 24)

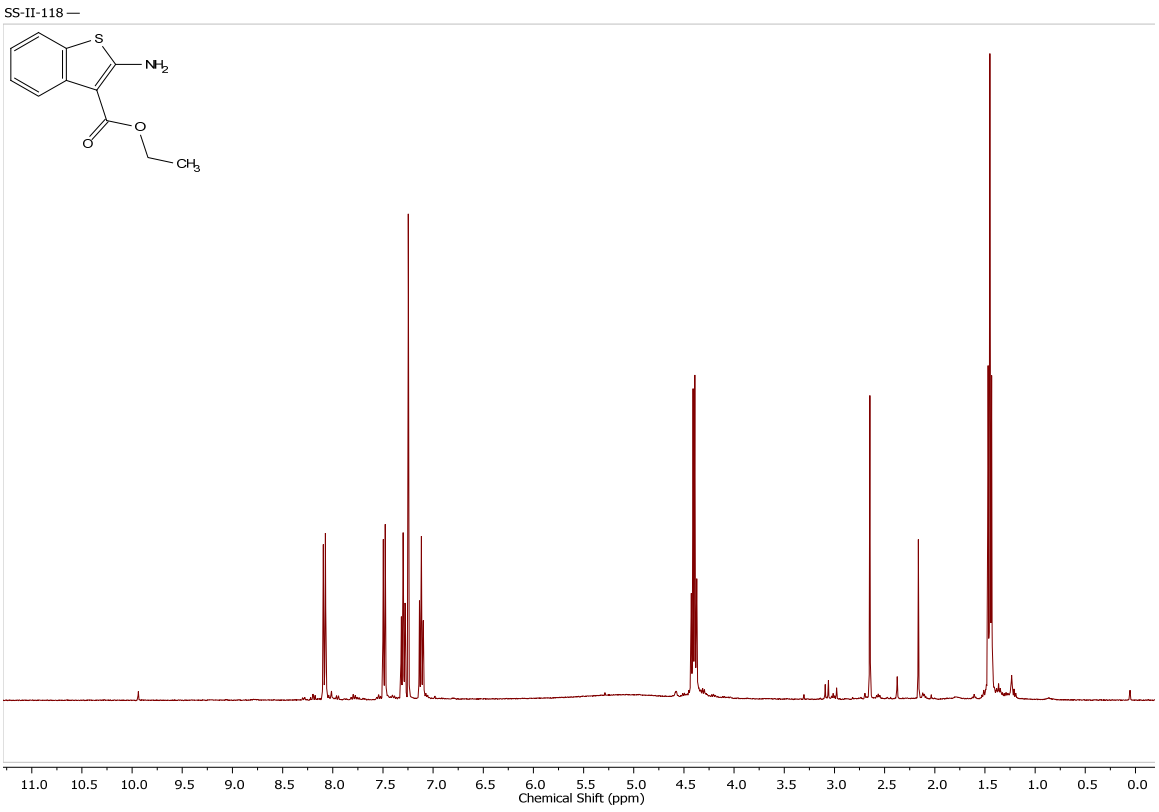
To an oven dry 15 mL round bottom flask were added amino ester **23** (1.09 g, 4.84 mmol) and dimethyl sulfoxide (DMSO) (5.0 mL, 0.968 M) and it was heated at 190 °C for 18 hours. The solution was cooled to room temperature and separation was completed using ethyl acetate and water, and the organic layer was dried with anhydrous sodium sulfate. Chromatography was completed using neutral aluminum oxide (20:80 mixture of ethyl acetate: hexane) in order to obtain aromatic amino ester **24** (0.0519 g, 0.235 mmol) at a 5% yield of an orange solid.

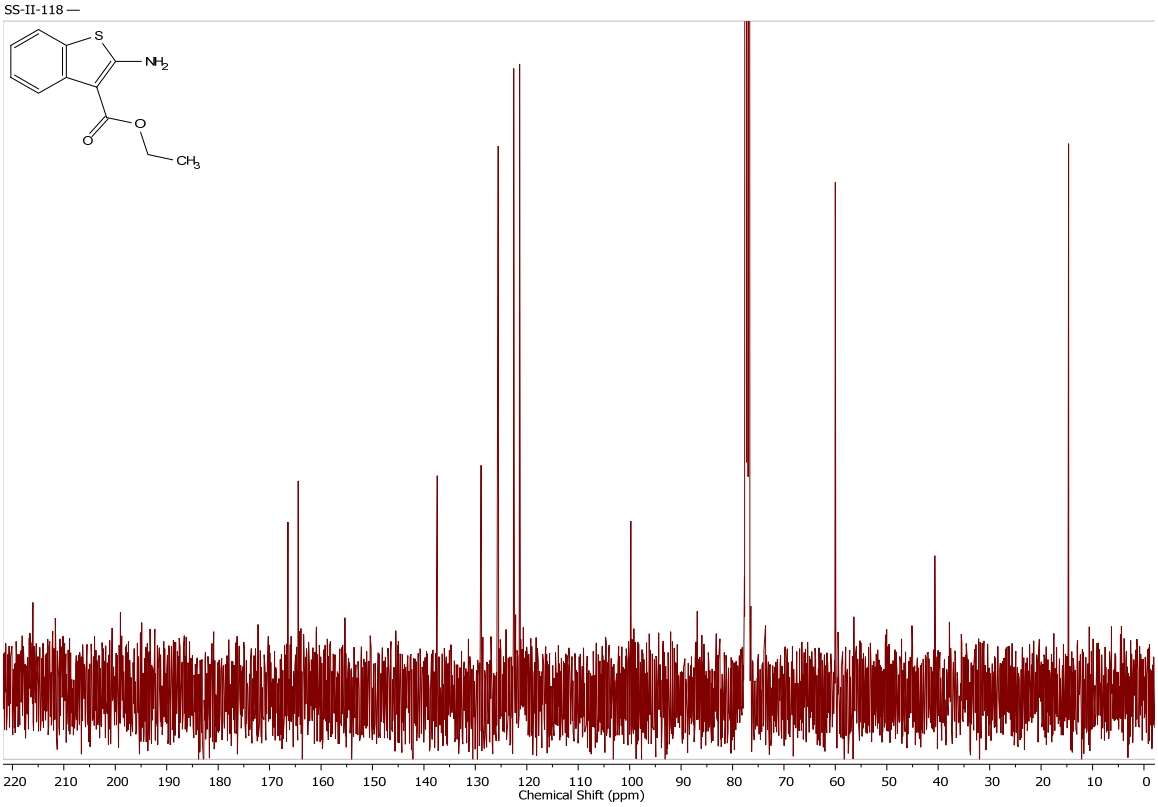


24

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 8.2 Hz, 1H), 7.49 (d, *J* = 7.8 Hz, 1H), 7.30 (t, *J* = 7.6 Hz, 1H), 7.12 (t, *J* = 7.6 Hz, 1H), 4.40 (q, *J* = 7.3 Hz, 2H), 1.50 - 1.41 (m, 3H) ppm.

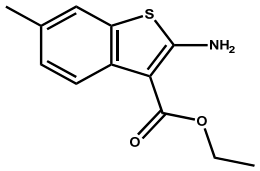
^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 164.2, 137.1, 128.7, 125.5, 122.5, 121.4, 100.1, 60.0, 40.9, 14.7 ppm.





Synthesis of ethyl 2-amino-6-methylbenzo[*b*]thiophene-3-carboxylate (aromatic amino ester 25)[15]

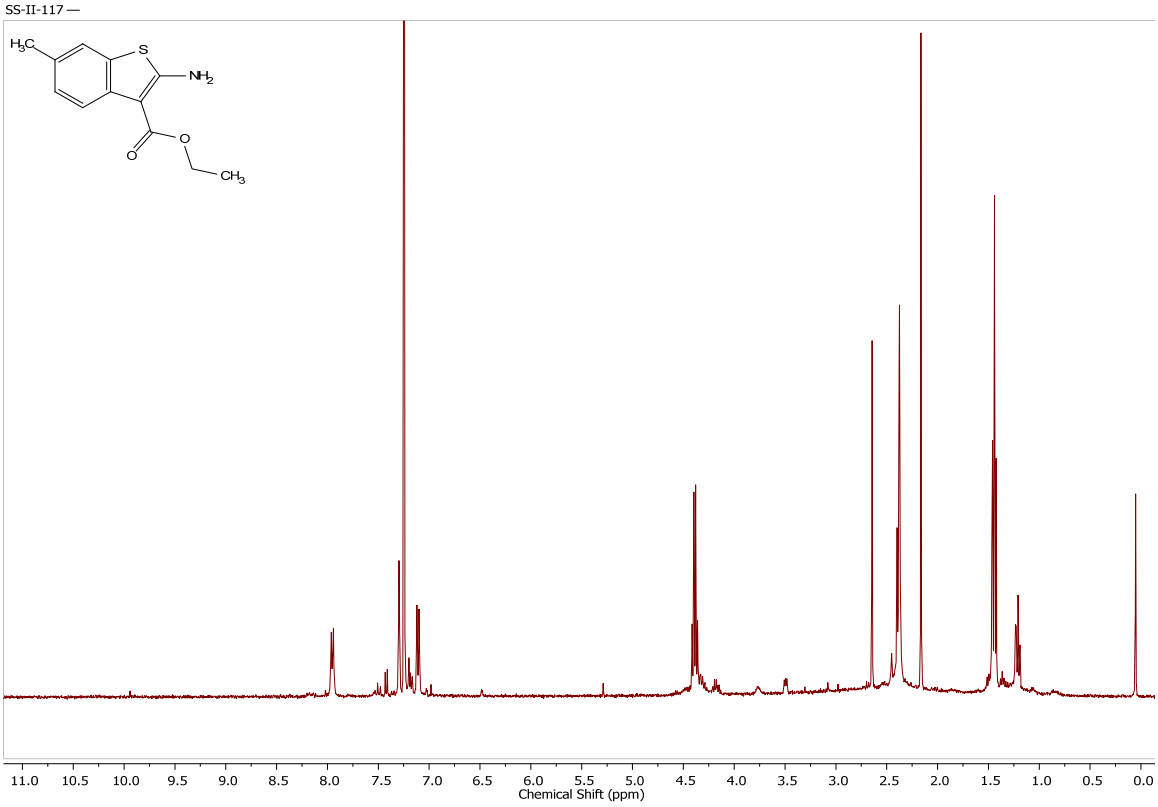
To an oven dry 15 mL round bottom flask were added amino ester **12** (1.13 g, 4.73 mmol) and dimethyl sulfoxide (DMSO) (5.0 mL, 0.946 M) and was heated at 190 °C for 18 hours, then reacted at room temperature for three days. Separation was completed using ethyl acetate and water, and the organic layer was dried with anhydrous sodium sulfate. Chromatography was completed using neutral aluminum oxide (20:80 mixture of ethyl acetate: hexane) in order to obtain aromatic amino ester **25** (0.350 g, 1.48 mmol) at a 31% yield of an orange solid.



25

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.2 Hz, 1 H), 7.30 (s, 1 H), 7.11 (d, *J* = 8.2 Hz, 1 H), 4.39 (q, *J* = 7.2 Hz, 2 H), 2.42 - 2.34 (m, 3 H), 1.49 - 1.40 (m, 3 H) ppm.

FTMS ESI: $C_{12}H_{13}NO_2S$ $[M+H]^+$, Calculated: 236.3090 g/mol, Found: 236.0736 g/mol.



3.4 Synthesis and Characterization of Pyrimidinone Derivatives

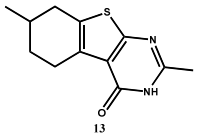
Pyrimidinone **13** and **19** were synthesized using the same reaction conditions. However, pyrimidinones **26** and **27** were synthesized using different conditions. Precaution concentrated hydrochloric acid is used for these reaction conditions and is extremely corrosive.

Synthesis of 2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (Pyrimidinone 13).[11]

Amino ester (**12**) (0.564 g, 2.10 mmol) was obtained and reacted in an oven dry microwave vial. Hydrochloric acid (1.40 mL, 16.7 mmol, 12.1 M) and acetonitrile (1.24 mL, 38.4 mmol) were added to the vial under inert atmosphere. The reaction was then placed in the microwave at 100 °C for 75 minutes. The product precipitated out of solution and is obtained through a filtration using cold water. There was a 65% yield of (pyrimidinone **13**) as a white solid (0.36 g, 1.54 mmol).

Synthesis of 2,7,7-trimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (Pyrimidinone 19).[11]

The synthesis of pyrimidinone **19**, used amino ester **19** (0.20 g, 0.79 mmol), under the same conditions mentioned above (synthesis of pyrimidinone **13**). Pyrimidinone **19** was synthesized in 80% yield, as an off white solid (0.152 g, 0.613 mmol), which was confirmed by ^{1}H NMR.

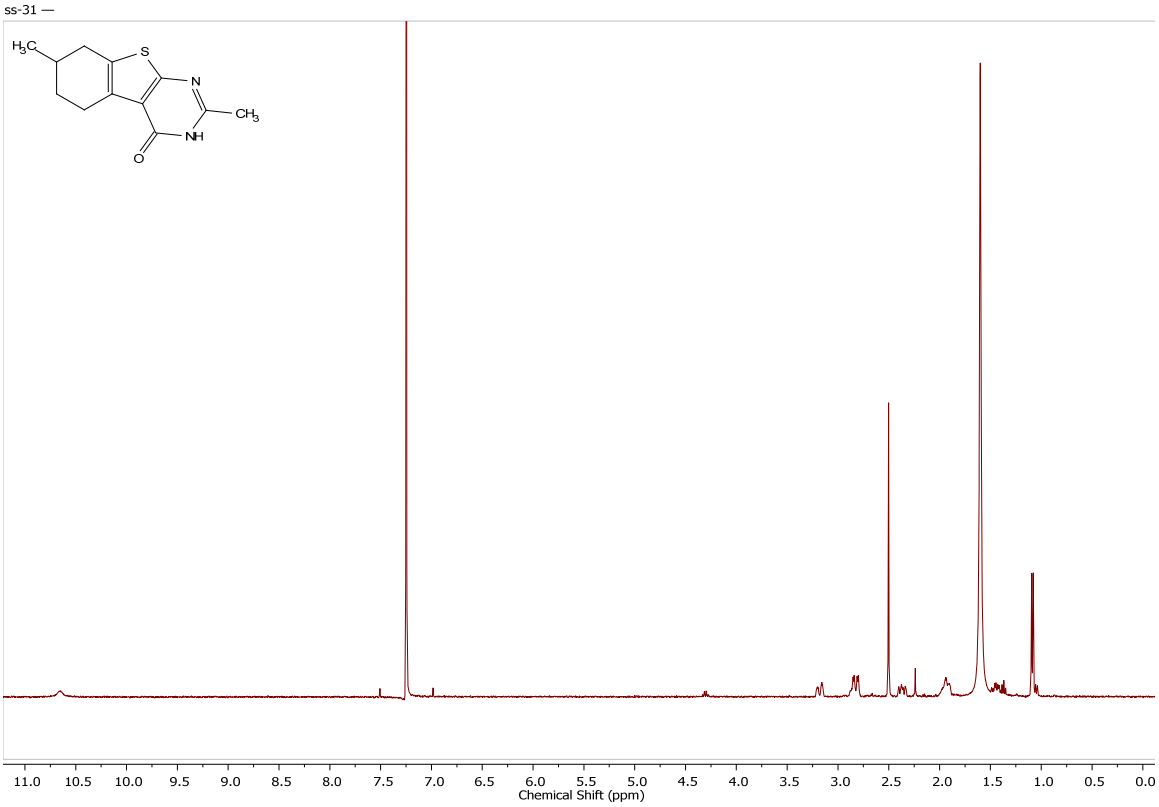


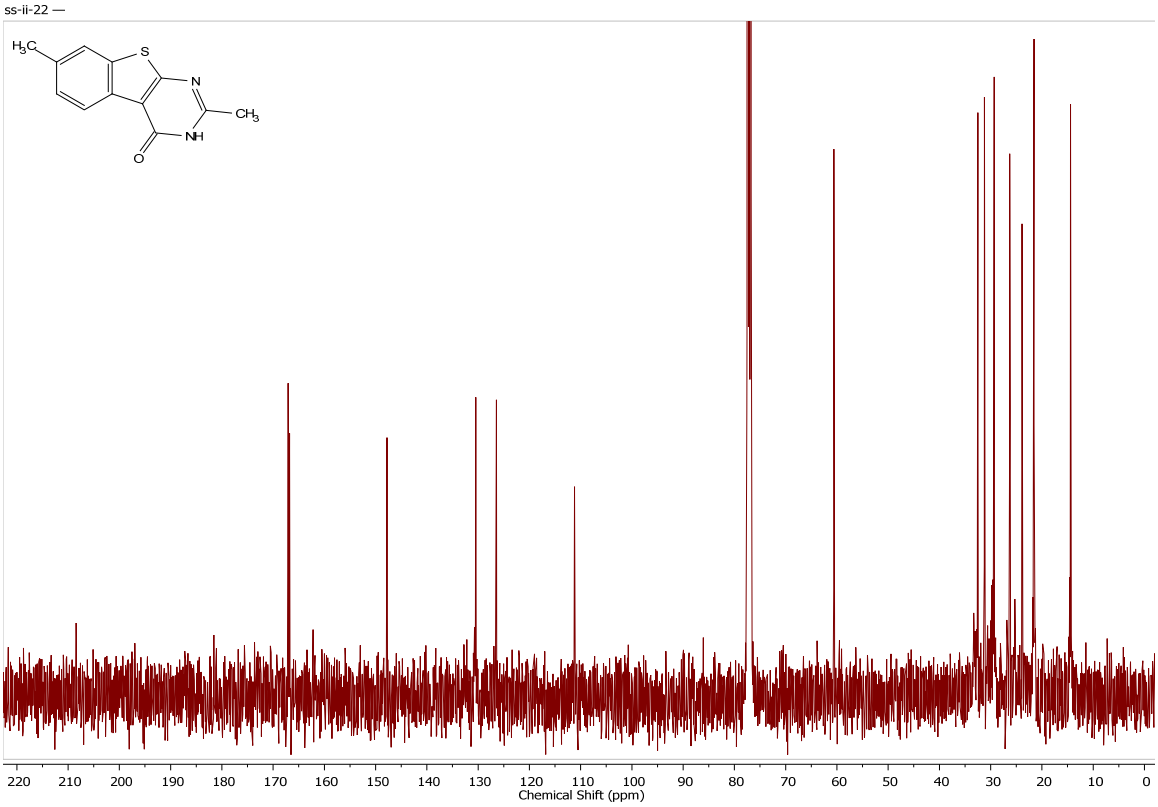
13

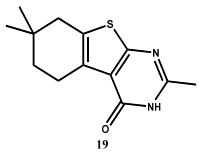
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.78 - 10.55 (m, 1H), 3.24 - 3.10 (m, 1H), 2.91 - 2.78 (m, 2H), 2.50 (s, 3H), 2.43 - 2.31 (m, 1H), 2.04 - 1.87 (m, 2H), 1.50 - 1.39 (m, 1H), 1.09 (d, *J* = 6.4 Hz, 2H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 147.8, 130.4, 126.4, 60.6, 32.5, 31.2, 29.3, 26.3, 23.9, 21.5, 14.4 ppm.

FTMS ESI: $C_{12}H_{15}N_2OS$ $[M+H]^+$, Calculated: 235.0905 g/mol, Found: 235.0895 g/mol.

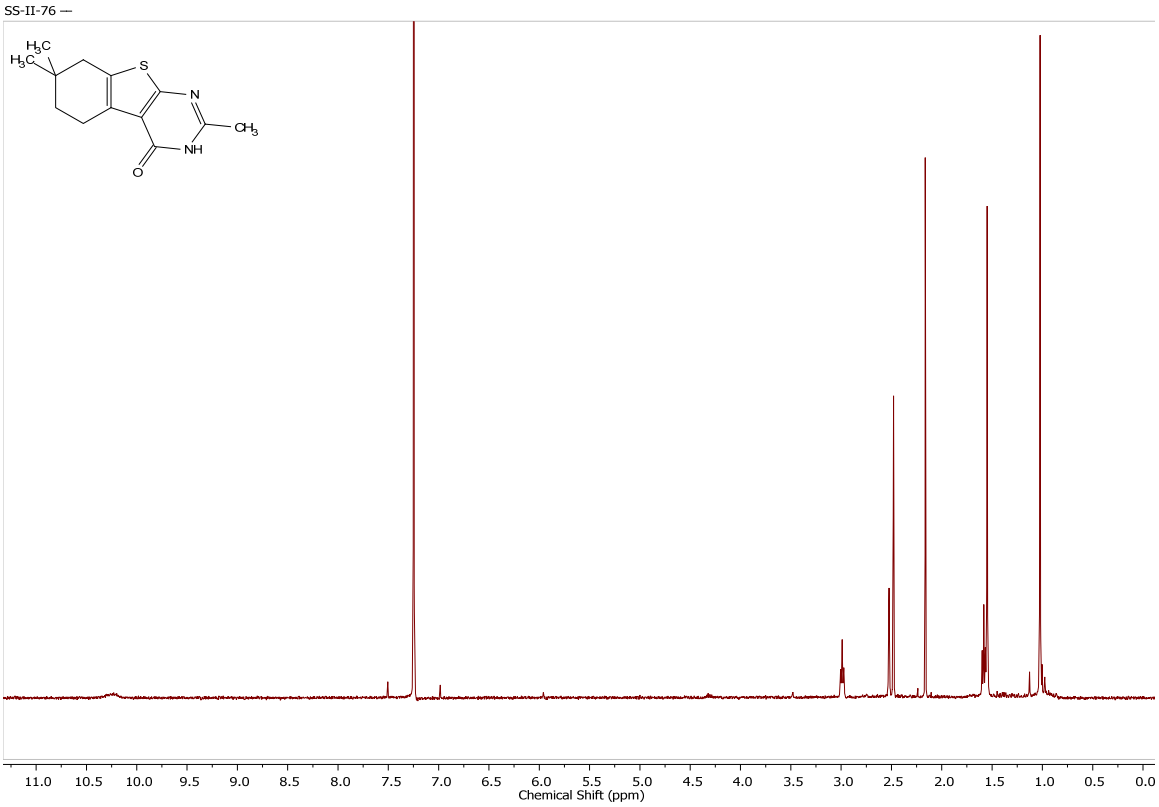

ss-31 —
H3C
S
N
CH3
NH
O
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Chemical Shift (ppm)





19

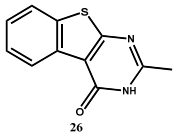
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.47 - 10.04 (m, 1H), 3.08 - 2.90 (m, 2H), 2.52 (s, 2H), 2.16 (s, 3H), 1.58 (s, 2H), 1.02 (s, 6H) ppm.



Synthesis of 2-methylbenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (Pyrimidinone 26).[11]

Amino ester (**24**) (0.052 g, 0.232 mmol) was reacted in an oven dry microwave vial with hydrochloric acid (0.20 mL, 2.42 mmol, 12.1 M) and acetonitrile (0.4 mL, 7.66 mmol) under inert atmosphere. The reaction was then sealed and heated in the microwave at 100 °C for 75 minutes. The product precipitated out of solution and is obtained through a filtration using cold water. Chromatography (using initially hexane, then ethyl acetate) was completed in order to obtain pyrimidinone **26** (0.019 g, 0.089 mmol) at a 37% yield of a tan solid.

Synthesis of 2,7-dimethylbenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (Pyrimidinone 27).[11]Amino ester (**24**) (0.172 g, 0.722 mmol) was reacted in an oven dry microwave vial with hydrochloric acid (0.45 mL, 5.45 mmol, 12.1 M) and acetonitrile (0.8 mL, 15.3 mmol) under inert atmosphere. The reaction was then sealed and heated in the microwave at 100 °C for 75 minutes. The product precipitated out of solution and is obtained through a filtration using cold water. Chromatography (5:95 mixture of ethyl acetate: hexane) was completed in order to obtain pyrimidinone **27** (0.034 g, 0.144 mmol) at a 24% yield of a tan solid.

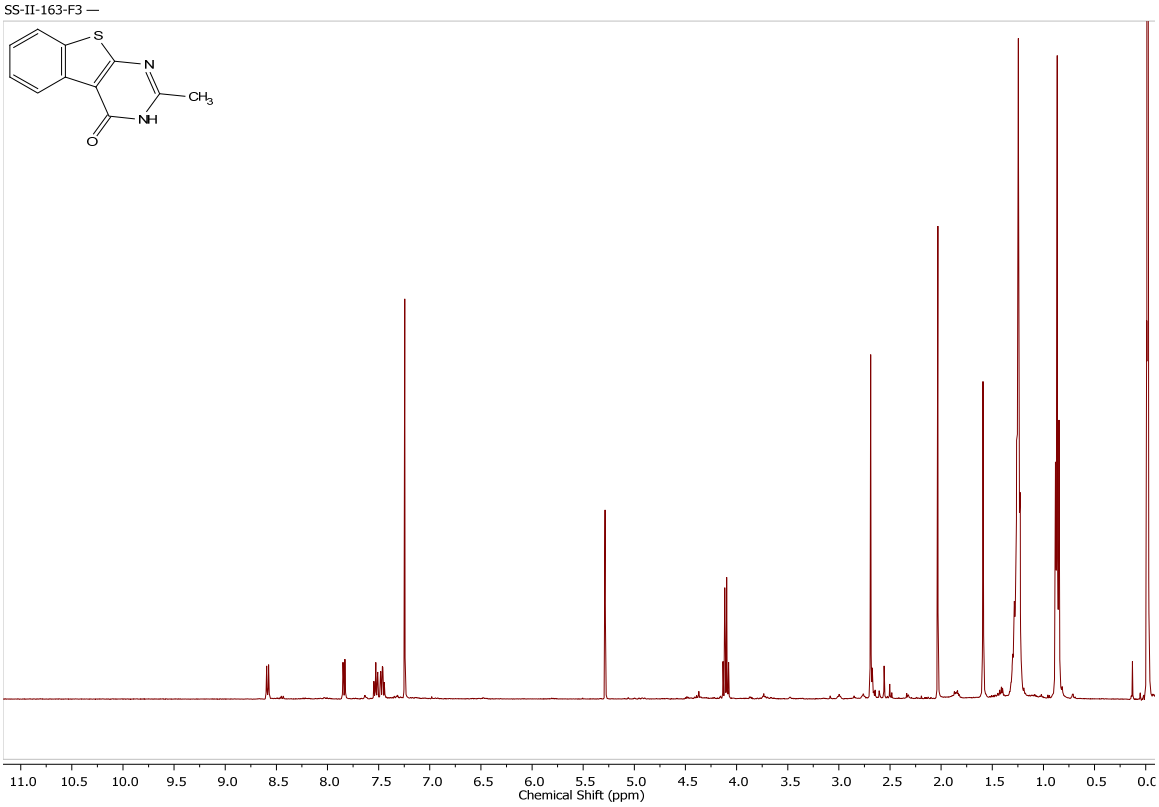


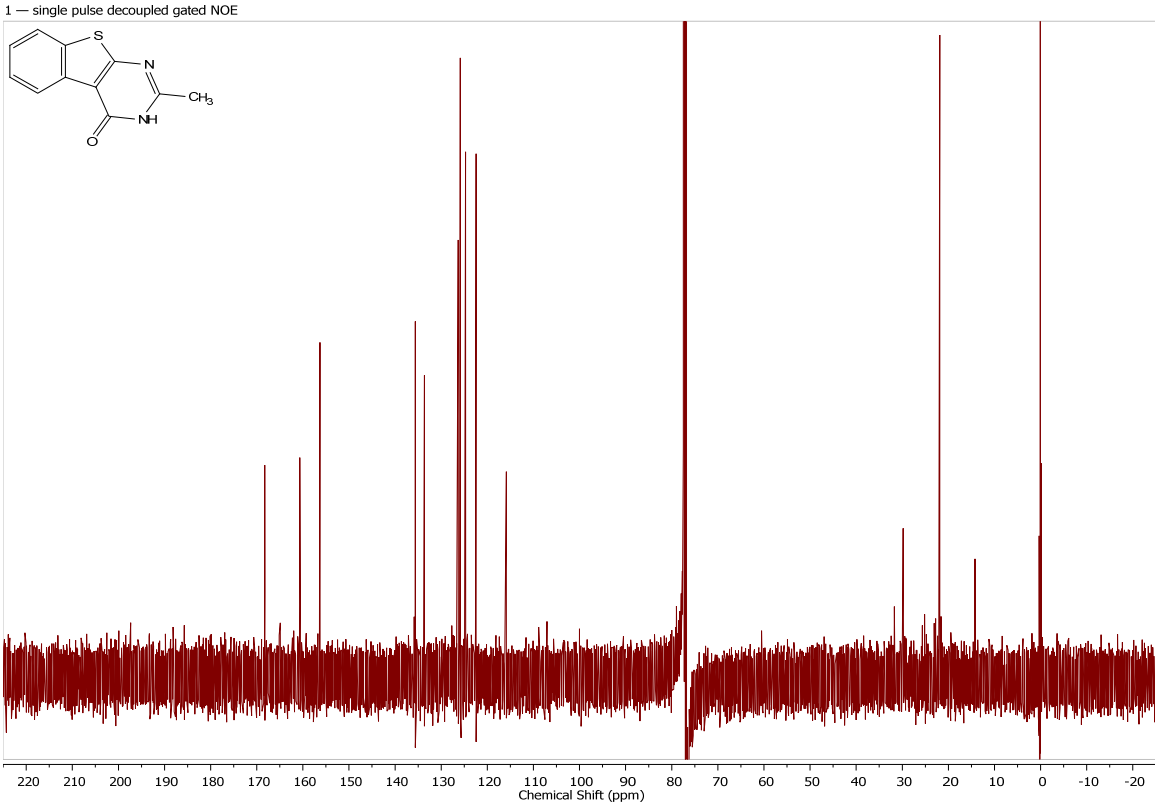
26

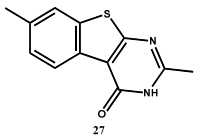
^{1}H NMR (500 MHz, $CDCl_3$) δ 12.66 - 12.12 (m, 1H), 8.66 - 8.50 (m, 1H), 7.90 - 7.80 (m, 1H), 7.61 - 7.51 (m, 1H), 7.52 - 7.44 (m, 1H), 2.69 (s, 3H) ppm.

^{13}C NMR (125 MHz, $CDCl_3$) δ 168.0, 160.7, 156.0, 135.6, 133.7, 126.1, 124.7, 122.4, 115.8, 29.5, 21.9 ppm.

FTMS ESI: $C_{11}H_9N_2OS$ $[M+H]^+$, Calculated: 217.04 g/mol, Found: 217.0427 g/mol.


SS-II-163-F3 —
S
N
CH3
NH
O
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Chemical Shift (ppm)



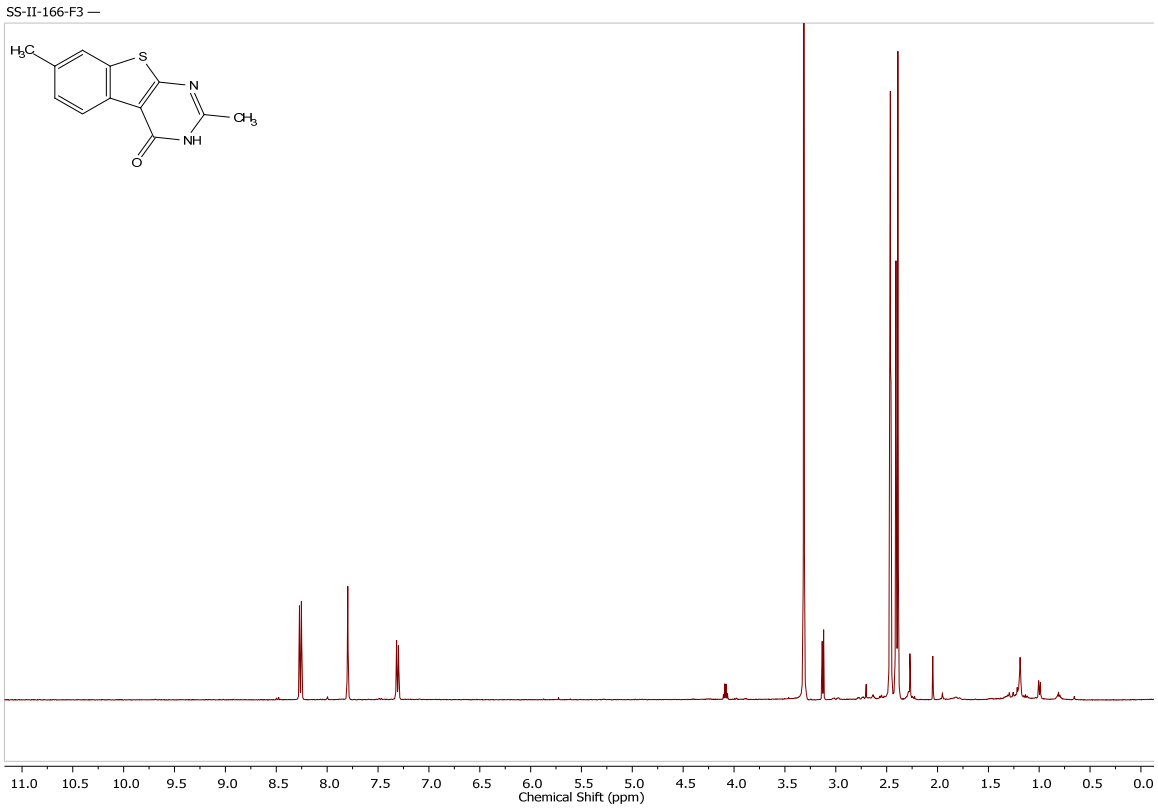


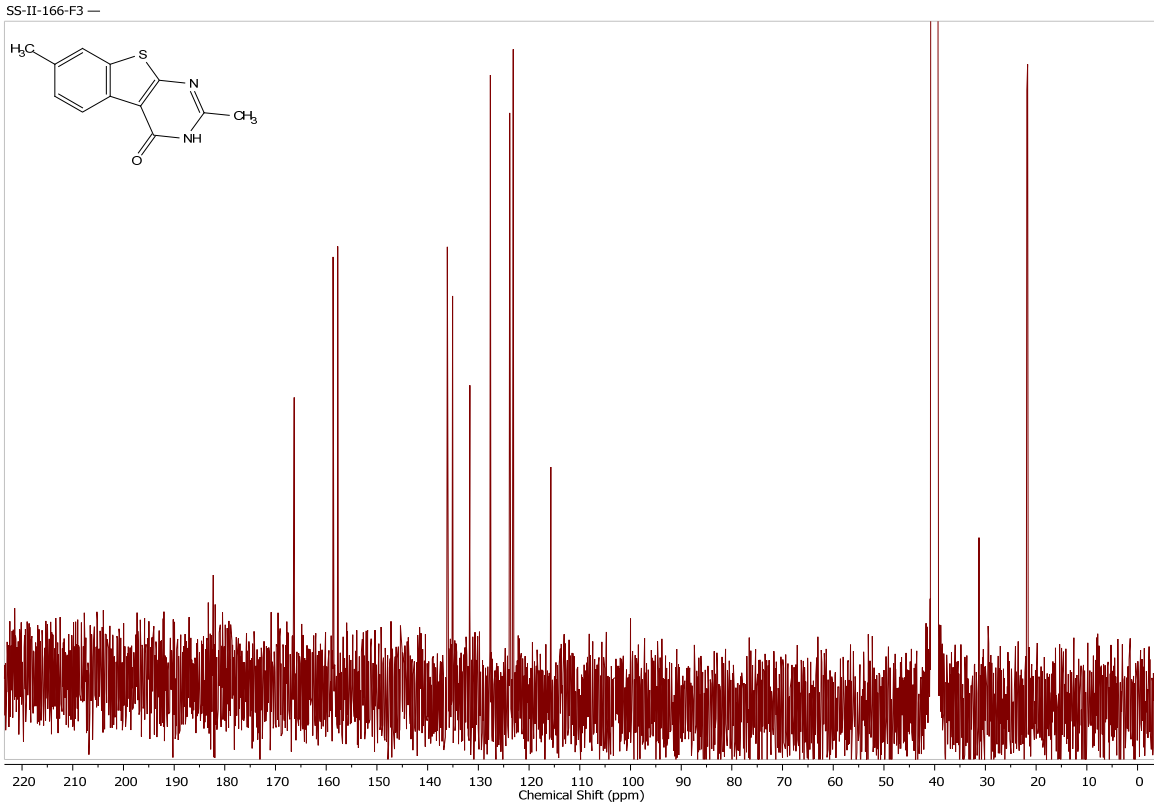
27

^{1}H NMR (400MHz ,DMSO-d$_6$) δ 8.26 (d, *J* = 7.8 Hz, 1H), 7.80 (s, 1H), 7.31 (dd, *J* = 0.9, 8.2 Hz, 1H), 2.41 (s, 3H), 2.39 (s, 3H) ppm.

^{13}C NMR (101MHz ,DMSO-d$_6$) δ 166.4, 159.0, 157.2, 136.3, 134.5, 131.4, 127.7, 124.1, 122.5, 115.7, 21.8, 21.6 ppm.

FTMS ESI: $C_{12}H_{11}N_2OS$ $[M+H]^+$, Calculated: 231.0592 g/mol, Found: 231.0583 g/mol.


SS-II-166-F3 —
H3C
S
N
CH3
NH
O
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Chemical Shift (ppm)



3.5 Synthesis and Characterization of Chloropyrimidine Derivatives [12]

The synthesis of chloropyrimidine derivatives (**14**, **21**, **28**, **29**, and **32**) was completed using the same procedure. Precautions for this reaction are $POCl_3$ is toxic and needs to be handled with care also when quenching the reaction with KOH it is exothermic use caution and cold KOH.

Synthesis of 4-chloro-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine (chloropyrimidine 14).[12]

The pyrimidinone (**13**; 0.190 g, 0.808 mmol) was reacted with $POCl_3$ (0.56 mL, 4.44 mmol) in the microwave for 3.5 hours at 150 °C. Separation was completed using cold aqueous 1M KOH and ethyl acetate, and the organic layer was dried with anhydrous

sodium sulfate. The product (**18**) is concentrated down and obtained at 94% yield as a red-orange solid (0.084 g, 0.33 mmol).

Synthesis of 4-chloro-2,7,7-trimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine (chloropyrimidine 21).[12]

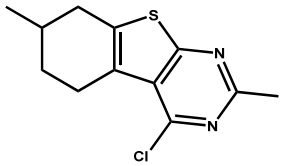
The synthesis of chloropyrimidine **21** used pyrimidinone **20** (0.024 g, 0.098 mmol), under the same conditions mentioned above (synthesis of chloropyrimidine **14**). Chloropyrimidine **21** was synthesized in a 93% yield, as a brown solid (0.024 g, 0.091 mmol).

Synthesis of 4-chloro-2-methylbenzo[4,5]thieno[2,3-d]pyrimidine (chloropyrimidine 26).[12]

The synthesis of chloropyrimidine **26** used pyrimidinone **24** (0.019 g, 0.087 mmol), under the same conditions mentioned above (synthesis of chloropyrimidine **14**). Chloropyrimidine **26** was synthesized in a 61% yield, as a yellow solid (0.012 g, 0.053 mmol).

Synthesis of 4-chloro-2,7-dimethylbenzo[4,5]thieno[2,3-d]pyrimidine (chloropyrimidine 27).[12]

The synthesis of chloropyrimidine **27** used pyrimidinone **25** (0.034 g, 0.149 mmol), under the same conditions mentioned above (synthesis of chloropyrimidine **14**). Chloropyrimidine **27** was synthesized in a 94% yield, as a brown solid (0.035 g, 0.141 mmol).

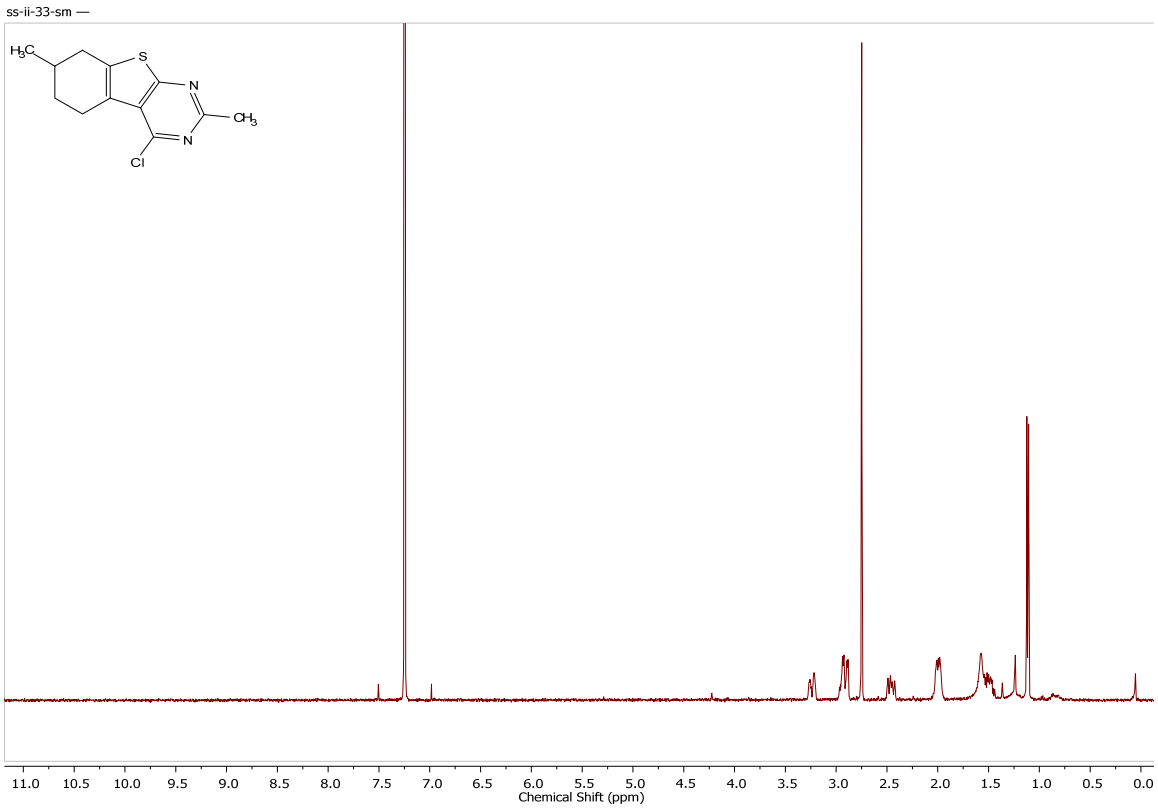


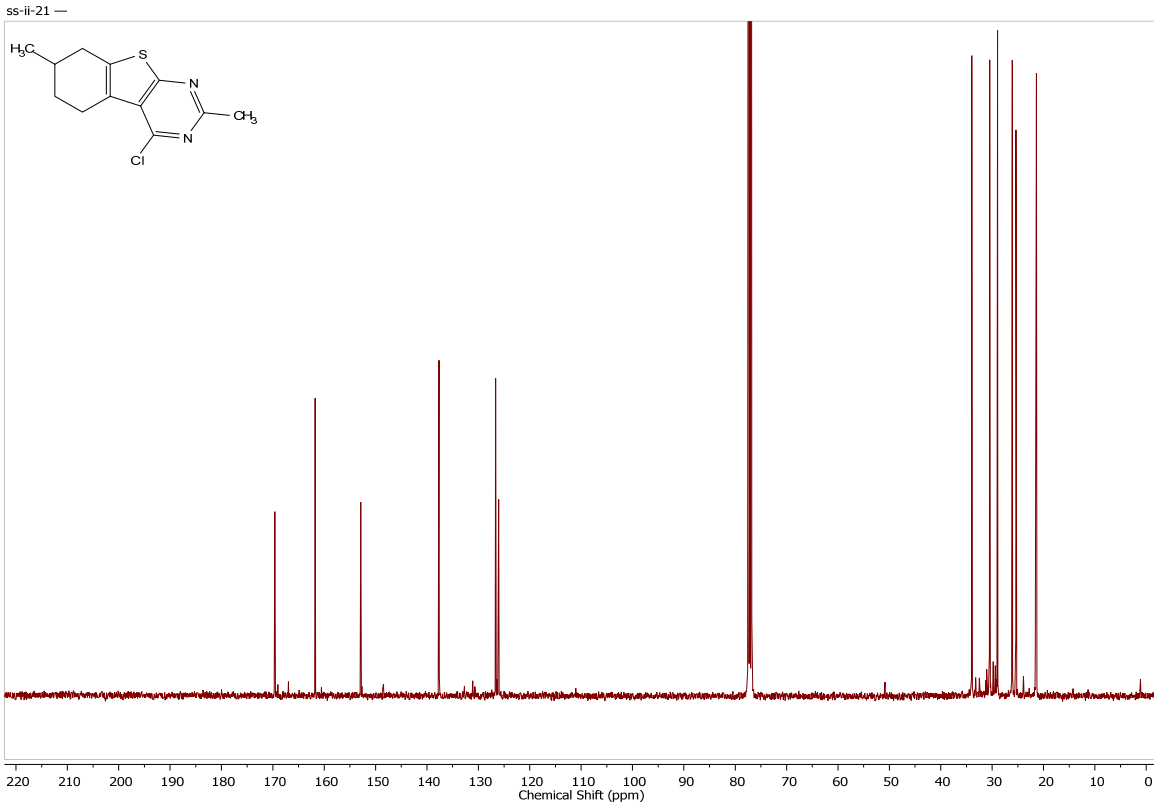
14

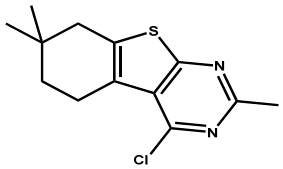
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.18 - 3.30 (m, 1H), 2.85 - 3.00 (m, 2H), 2.75 (s, 3H), 2.40 - 2.51 (m, 1H), 1.93 - 2.09 (m, 2H), 1.43 - 1.54 (m, 1H), 1.11 (d, J = 6.9 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 161.8, 152.9, 137.7, 126.6, 126.0, 33.9, 30.5, 29.0, 26.1, 25.4, 21.5 ppm.

FTMS ESI: $C_{12}H_{14}ClN_2S$ $[M+H]^+$, Calculated: 253.0566 g/mol, Found: 253.0556 g/mol.

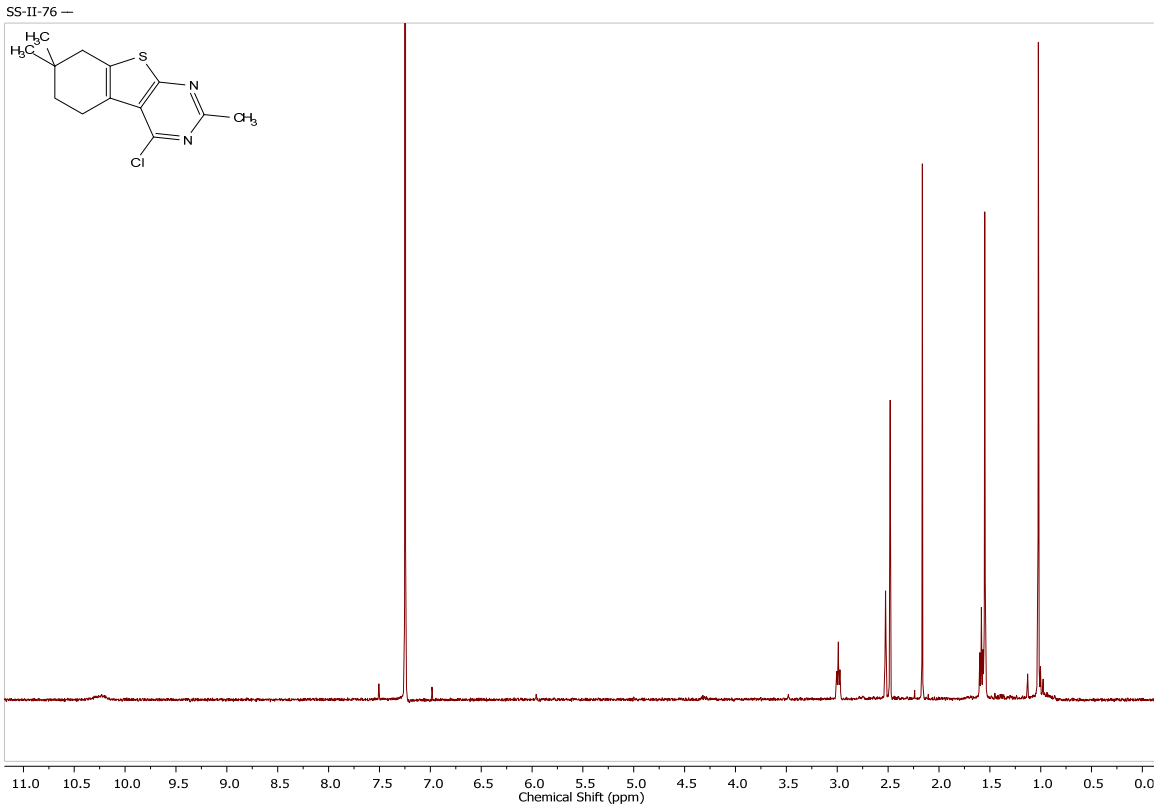

ss-ii-33-sm —
H3C
S
N
CH3
N
Cl
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Chemical Shift (ppm)

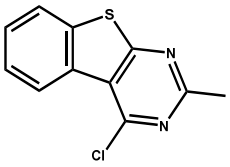




21

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.05 (t, *J* = 6.4 Hz, 2H), 2.76 (s, 3H), 2.61 (s, 2H), 1.64 (t, *J* = 6.6 Hz, 2H), 1.04 (s, 6H) ppm.



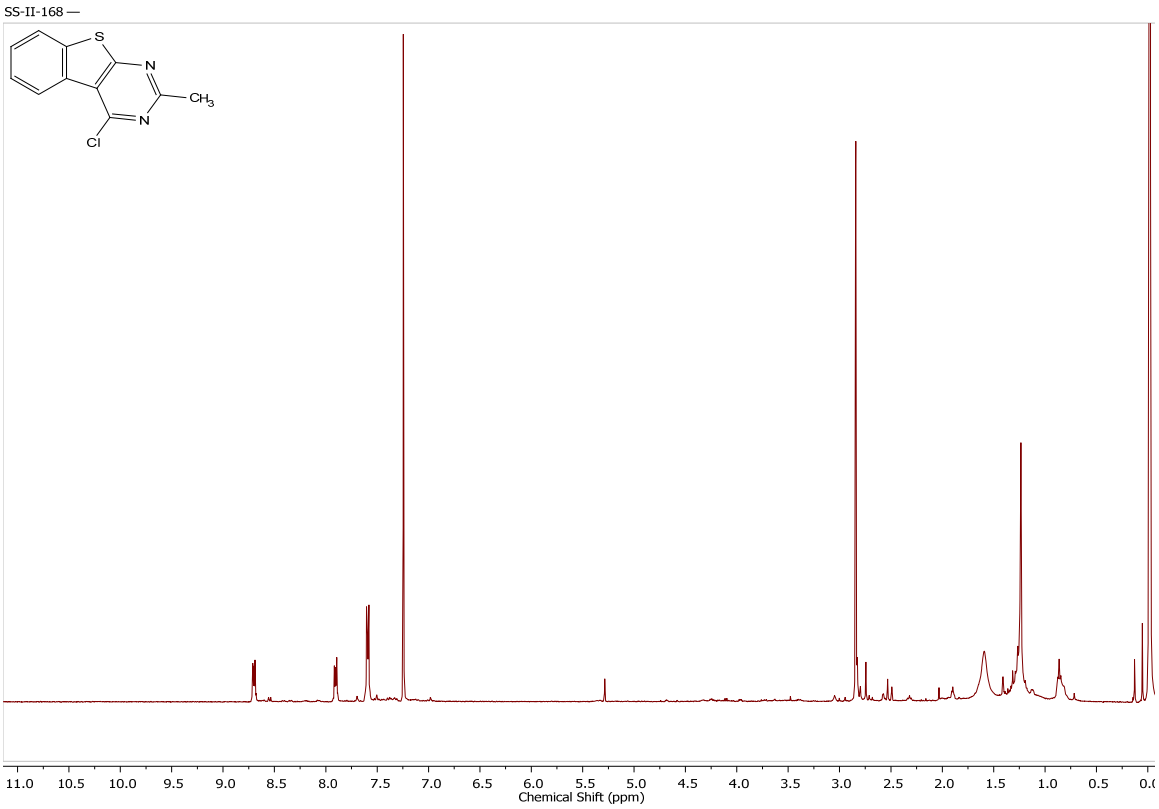


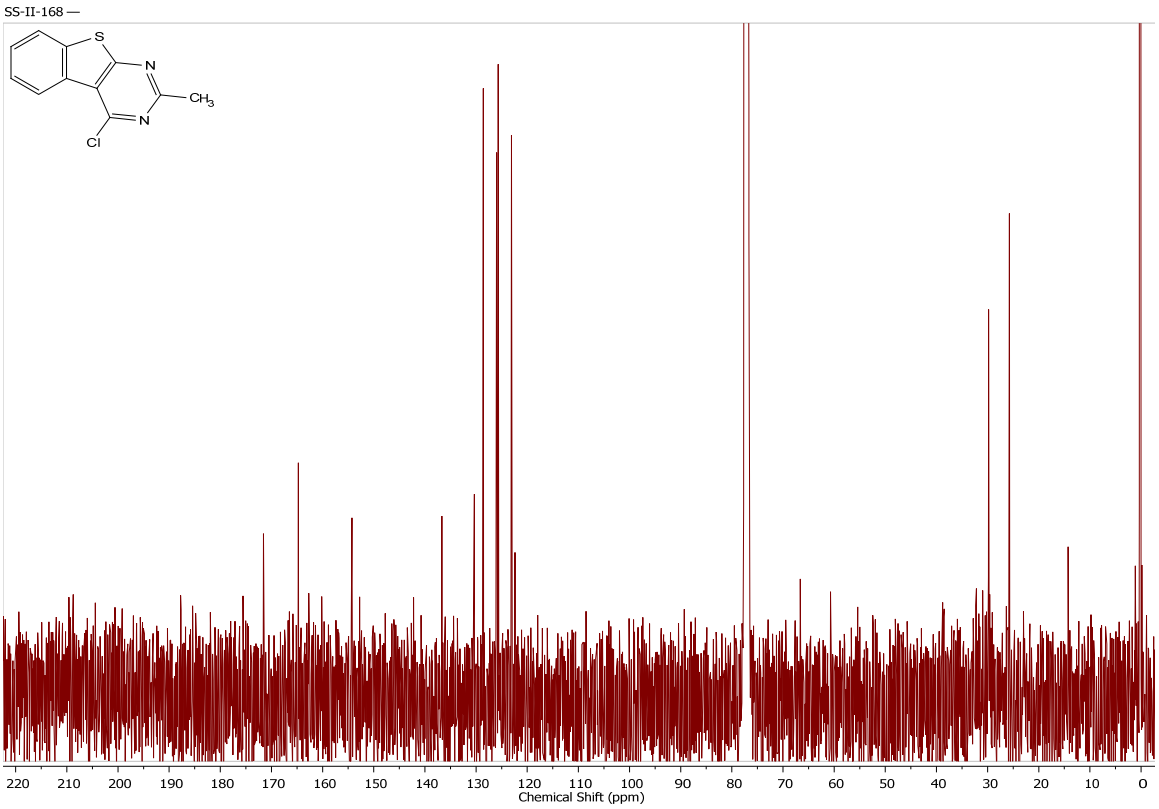
28

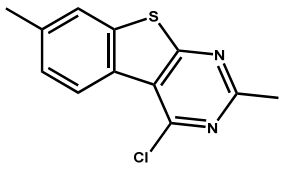
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 - 8.66 (m, 1H), 7.95 - 7.86 (m, 1H), 7.64 - 7.54 (m, 2H), 2.84 (s, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 164.3, 154.1, 136.3, 130.4, 128.2, 126.1, 125.6, 123.0, 122.3, 25.6 ppm.

FTMS ESI: $C_{11}H_8ClN_2S$ $[M+H]^+$, Calculated: 235.0097 g/mol, Found: 235.0089 g/mol.


SS-II-168
S
N
CH3
N
Cl
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Chemical Shift (ppm)



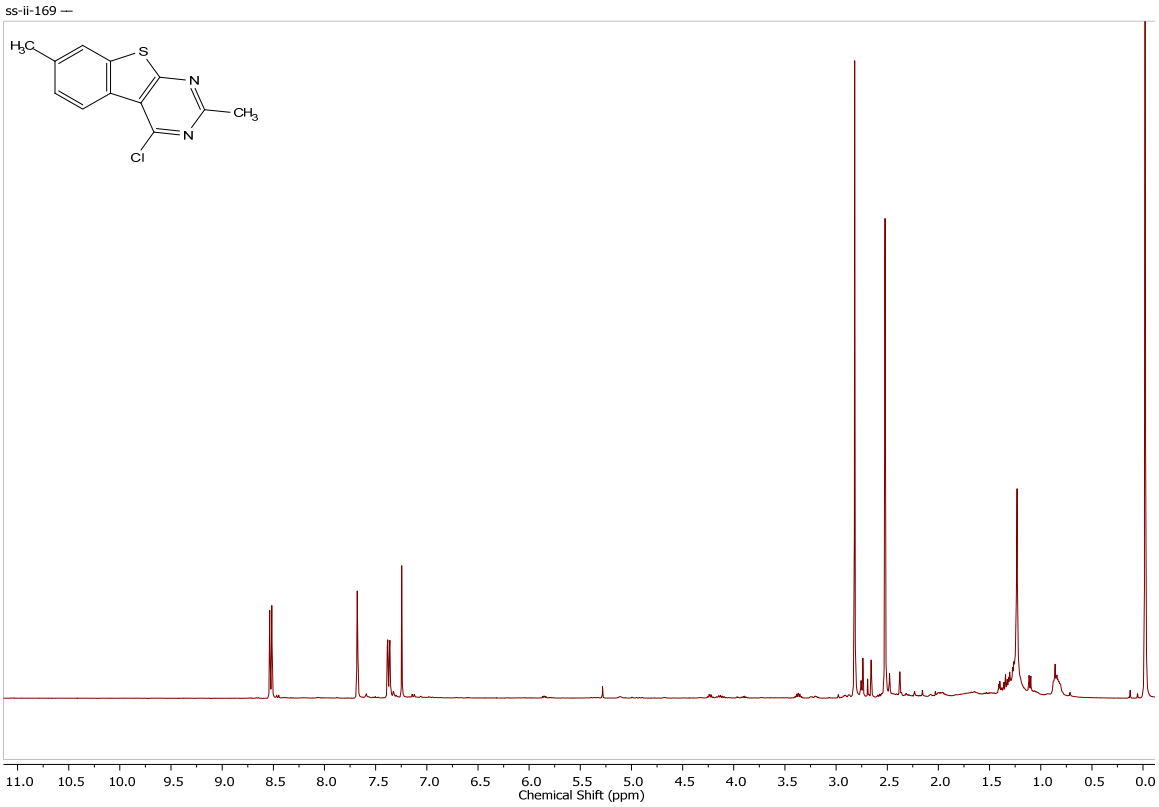


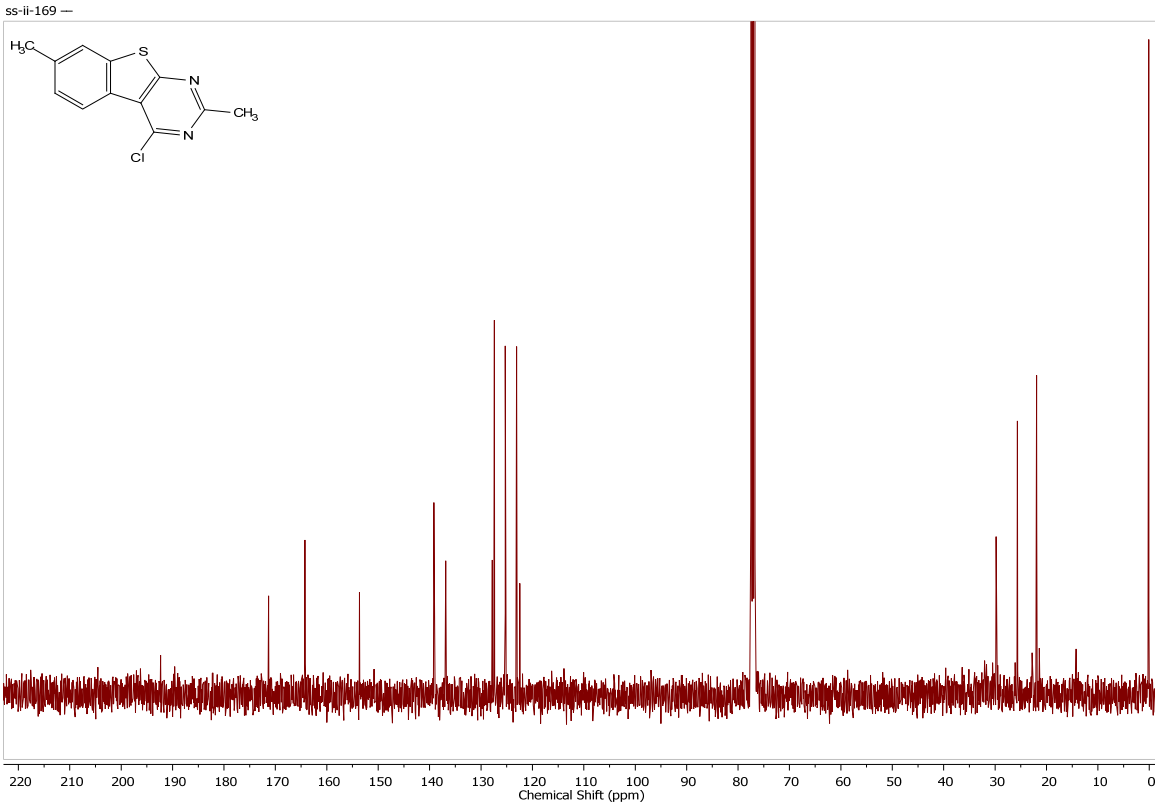
29

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J* = 8.2 Hz, 1H), 7.68 (s, 1H), 7.37 (d, *J* = 8.2 Hz, 1H), 2.82 (s, 3H), 2.52 (s, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 164.2, 153.7, 139.2, 136.9, 127.8, 127.2, 125.3, 123.1, 122.5, 25.7, 21.9 ppm.

FTMS ESI: $C_{13}H_{12}ClN_2S$ $[M+H]^+$, Calculated: 249.0253 g/mol, Found: 249.0244 g/mol.


ss-ii-169 —
H3C
S
N
CH3
N
Cl
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Chemical Shift (ppm)

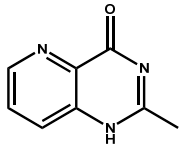


3.6 Synthesis and Characterization of Pyrido-[2,3-E]-Pyrimid-4-One

Synthesis of 2-methylpyrido[3,2-d]pyrimidin-4(1H)-one (Pyrido-[2,3-e]-pyrimid-4-one 31).[16]

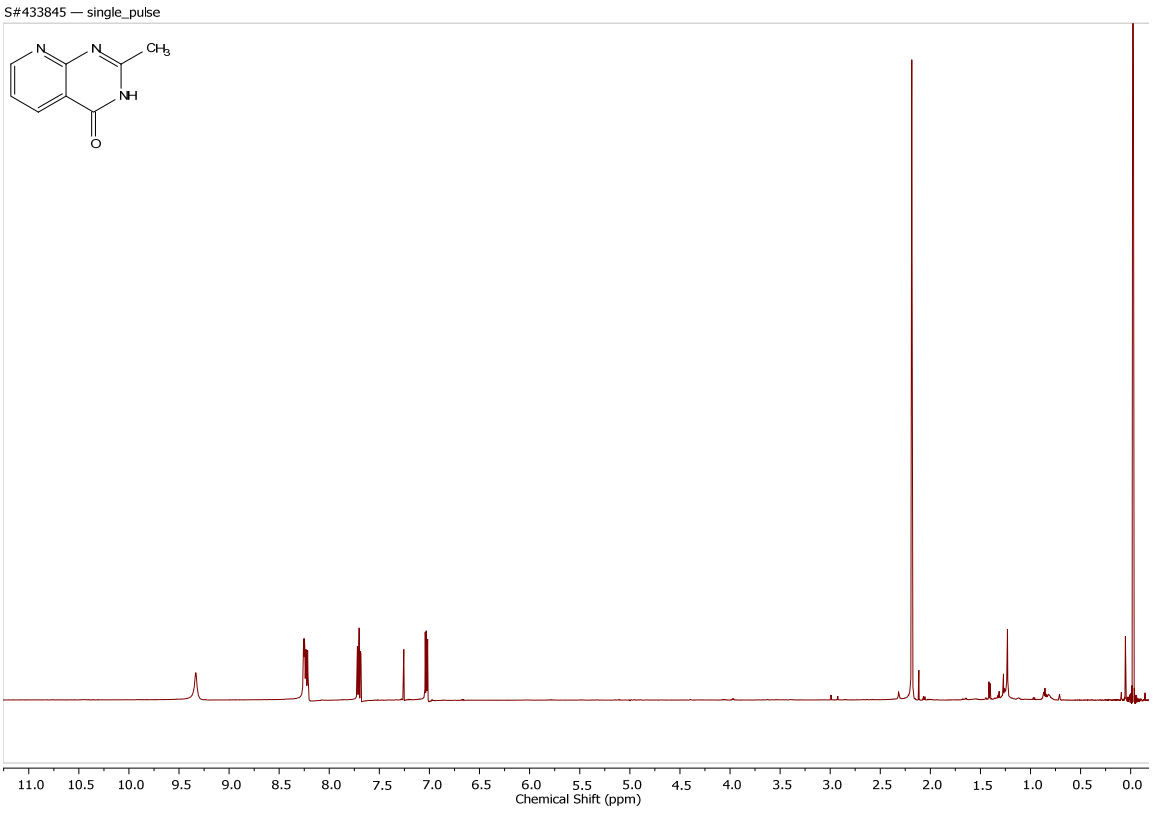
2-Aminonicotinic acid (0.050 g, 0.362 mmol), ammonium acetate (0.084 g, 1.09 mmol), acetic acid (0.16 mL, 1.45 mmol), and dimethylacetamide (DMA; 0.40 mL, 1.0 M) were added to an oven dry microwave vial (0.5-2.0 mL) under inert atmosphere. The reaction was then heated in the microwave at 250 °C for 30 minutes. Separation was completed using sodium chloride, water, and ethyl acetate and the organic layer was dried with anhydrous sodium sulfate. Chromatography (50:50 mixture ethyl acetate:

hexane) was completed in order to obtain pyrido-[2,3-e]-pyrimid-4-one **31** (0.078 g, 0.481 mmol), at a 28% yield of a yellow solid, which was confirmed by ^{1}H NMR.[16]



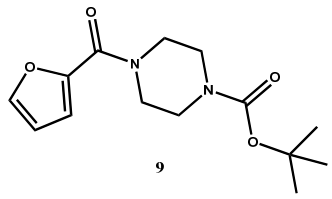
31

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (d, *J* = 2.3 Hz, 1H), 8.58 (dd, *J* = 1.6, 8.0 Hz, 1H), 7.48 - 7.37 (m, 1H), 2.64 (s, 3H) ppm.



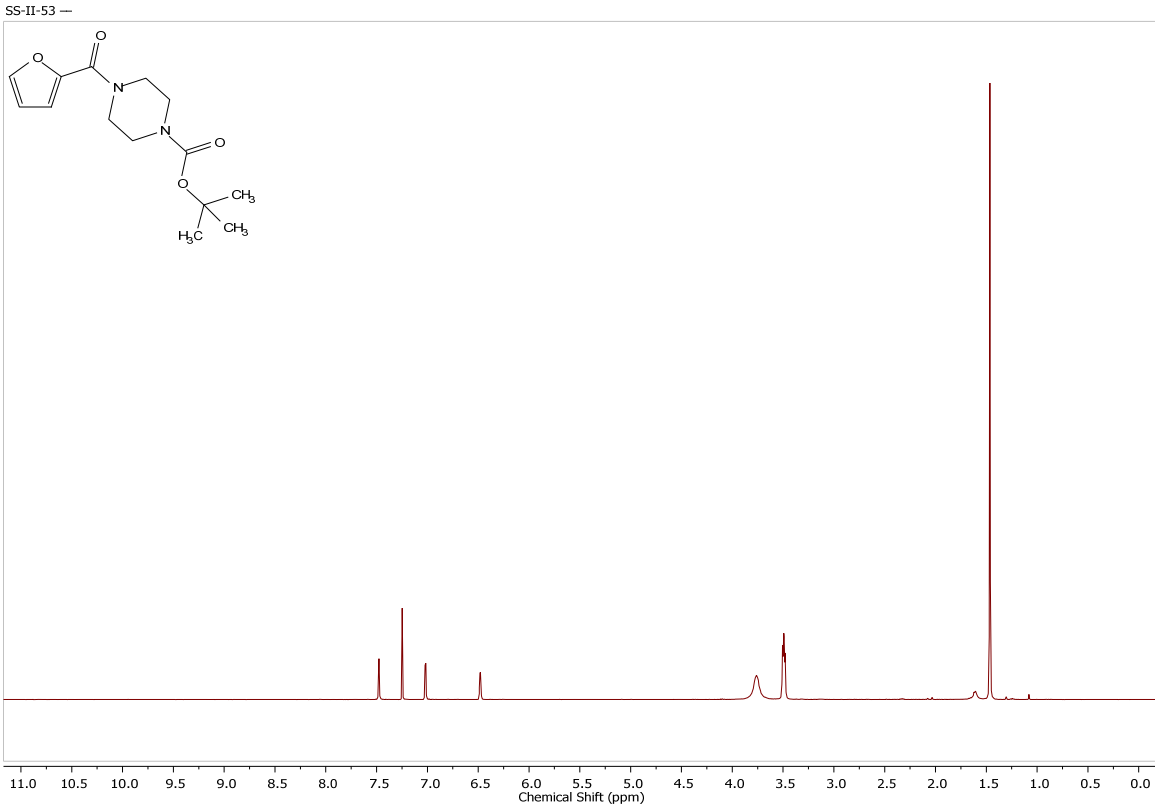
3.7 Synthesis and Characterization of Tert-Butyl 4-(Furan-2-Carbonyl)Piperzine-1-Carboxylate [8]

To a dry 50 mL round bottom flask was added 2-furylchloride (**9)** (1.67 mL, 16.8 mmol) 1-bocpiperizine (**10**) (3.14 g, 16.8 mmol), triethylamine (2.0 mL, 0.12 M), in 20 mL of DCM. The reaction was left to react 24 hours at room temperature and separation was completed using 1M hydrochloric acid and ethyl acetate. The product (**11**) was obtained with a 73% yield of a white solid (3.59 g, 12.8 mmol). The ^{1}H NMR data matched previously reported data.[8]



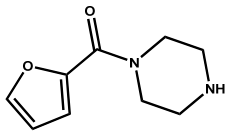
9

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.02 (d, *J* = 3.2 Hz, 1H), 6.48 (dd, *J* = 1.6, 3.4 Hz, 1H), 3.76 (br. s, 4H), 3.55 - 3.42 (m, 4H), 1.46 (s, 9H) ppm.


SS-II-53 —
O
O
N
N
O
O
CH3
H3C
CH3
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Chemical Shift (ppm)

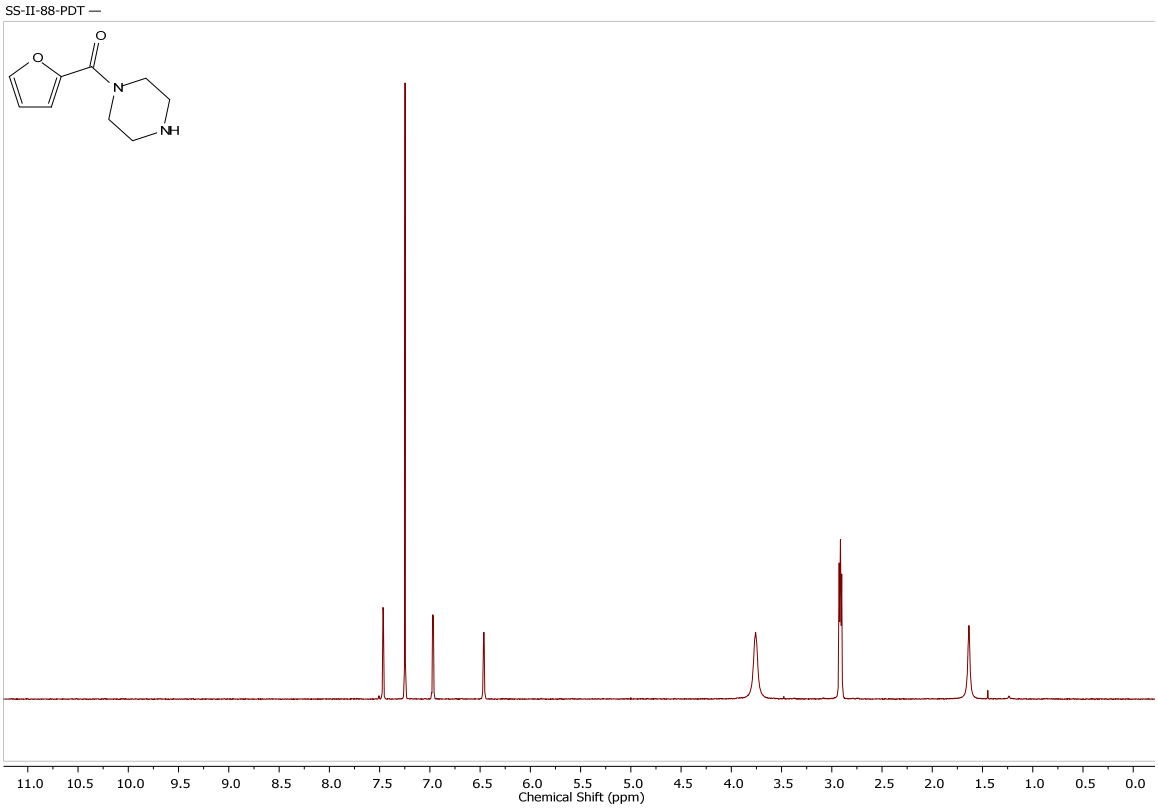
3.8 Synthesis and Characterization of Furan-2-Yl(Piperazin-1-Yl)Methanone [9]

To a small microwave vial was added 200 mg of furyl boc protected piperazine (**11)** and heated using a heat gun for 10 minutes for desired bubbling. Chromatography was completed (50% ethyl acetate and 50% methanol) which gave a 76% yield of a brown oil (0.165, 0.89 mmol), the deprotected boc piperazine product (**10**). The ^{1}H NMR data matched previously reported data.[9]



10

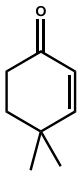
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 - 7.43 (m, 1H), 6.97 (d, *J* = 3.2 Hz, 1H), 6.46 (dd, *J* = 1.8, 3.7 Hz, 1H), 3.76 (br. s., 4H), 3.10 - 2.76 (m, 4H), 1.77 (br. s, 1 H) ppm.



3.9 Synthesis and Characterization of Cyclohexanone Derivative [13, 14]

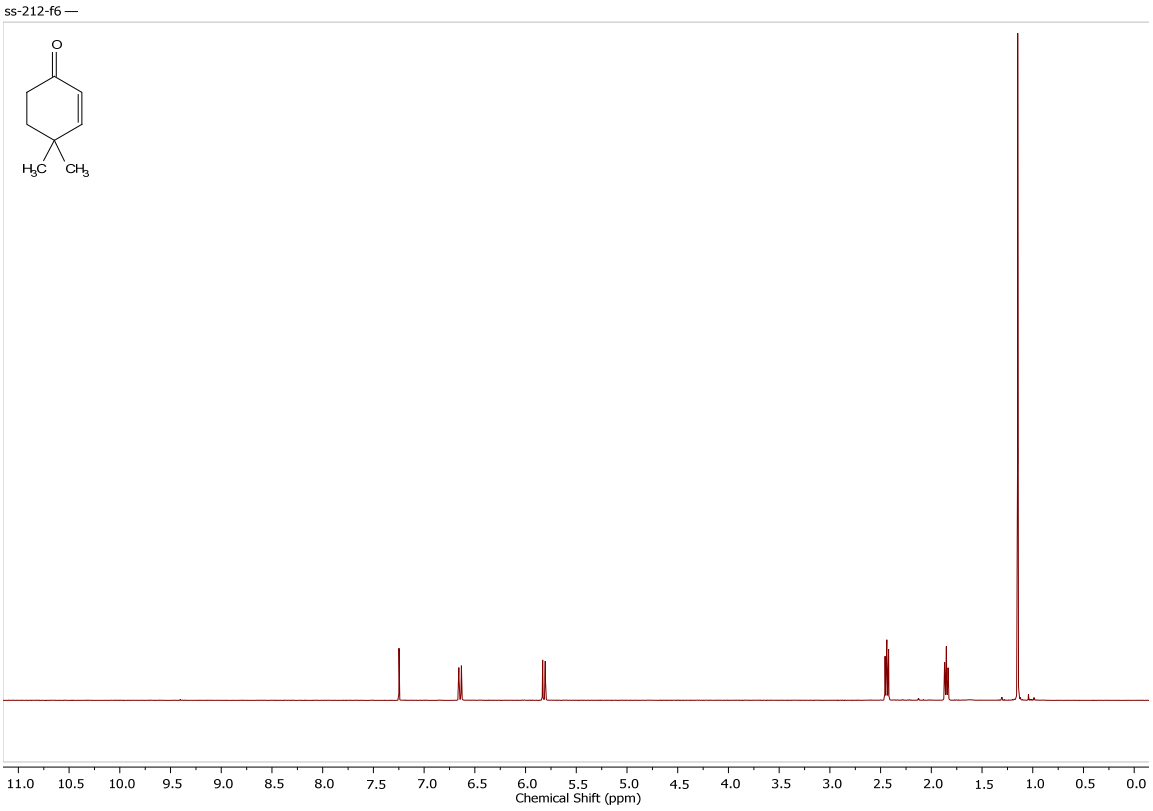
Synthesis of 4,4-dimethylcyclohex-2-ene-1-one.[13, 14]

A neat reaction of isoburtylaldehye (**15**; 10.5 mL, 166.8 mmol) and methyl vinyl ketone (**16**; 9.5 mL, 114.41 mmol) was reacted with sulfuric acid (0.05 mL). The reaction was run in a dean stark trap and a yellow oil; 4,4-dimethylcyclohex-2-ene-1-one (**17**; 12.4 g, 99.8 mmol) was obtained through short distant vacuum distillation at a 45% yield. The ^{1}H NMR data matched previously reported data.[13, 14]



17

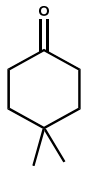
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.64 (d, *J* = 10.1 Hz, 1H), 5.82 (d, *J* = 10.1 Hz, 1H), 2.58 - 2.34 (m, 2H), 1.85 (t, *J* = 6.9 Hz, 2H), 1.15 (s, 6H) ppm.



Synthesis of 4,4, -dimethyl-cyclohex-2-ene-1-one.[13]

Caution do not place hydrogen atmosphere directly on palladium on carbon that is flammable, and methanol first. To a 100 mL RBF was added ketone (1.0 g, 64.5 mmol), palladium on carbon (0.020 g, 50% Pd) and dry methanol (5.38 mL, 1.4 M) under an

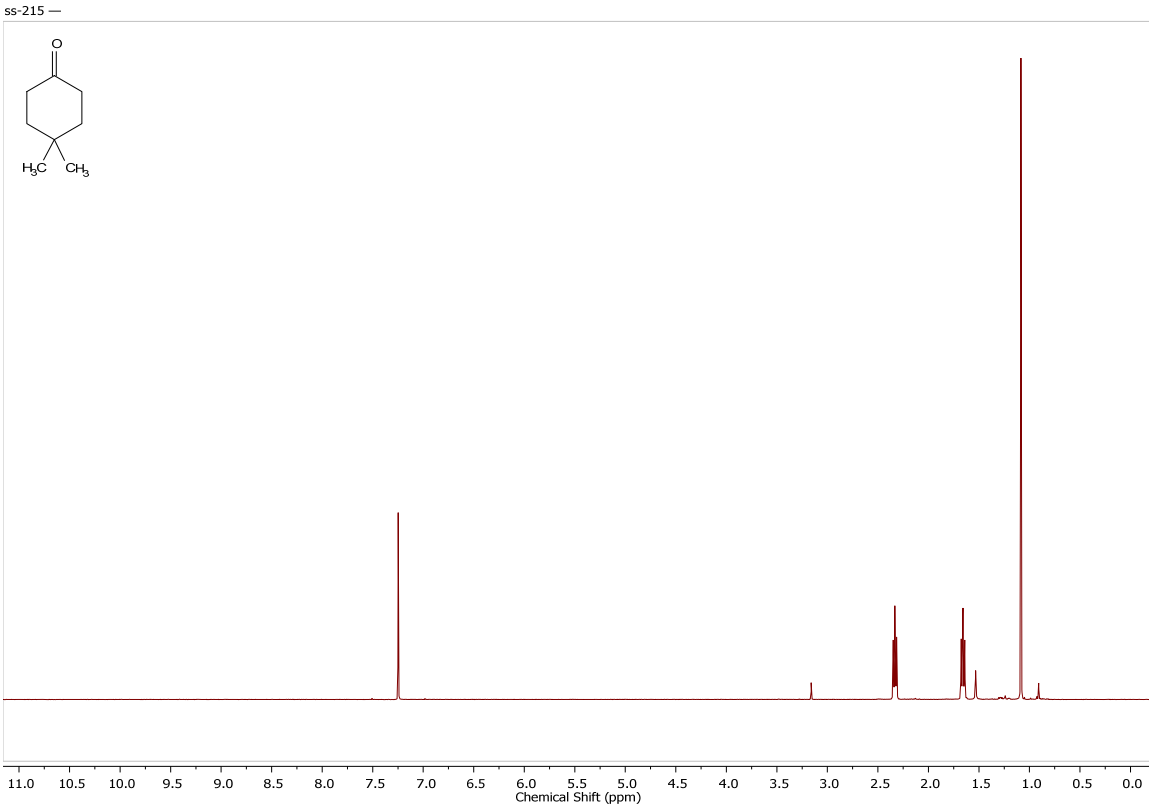
inert hydrogen gas atmosphere. The reaction then reacted for 24 hours at room temperature and was filtered through Celite. Ketone (**18**) was obtained at 90% yield of clear oil (0.92g, 7.25mmol). The ^{1}H NMR data matched previously reported data.[13]

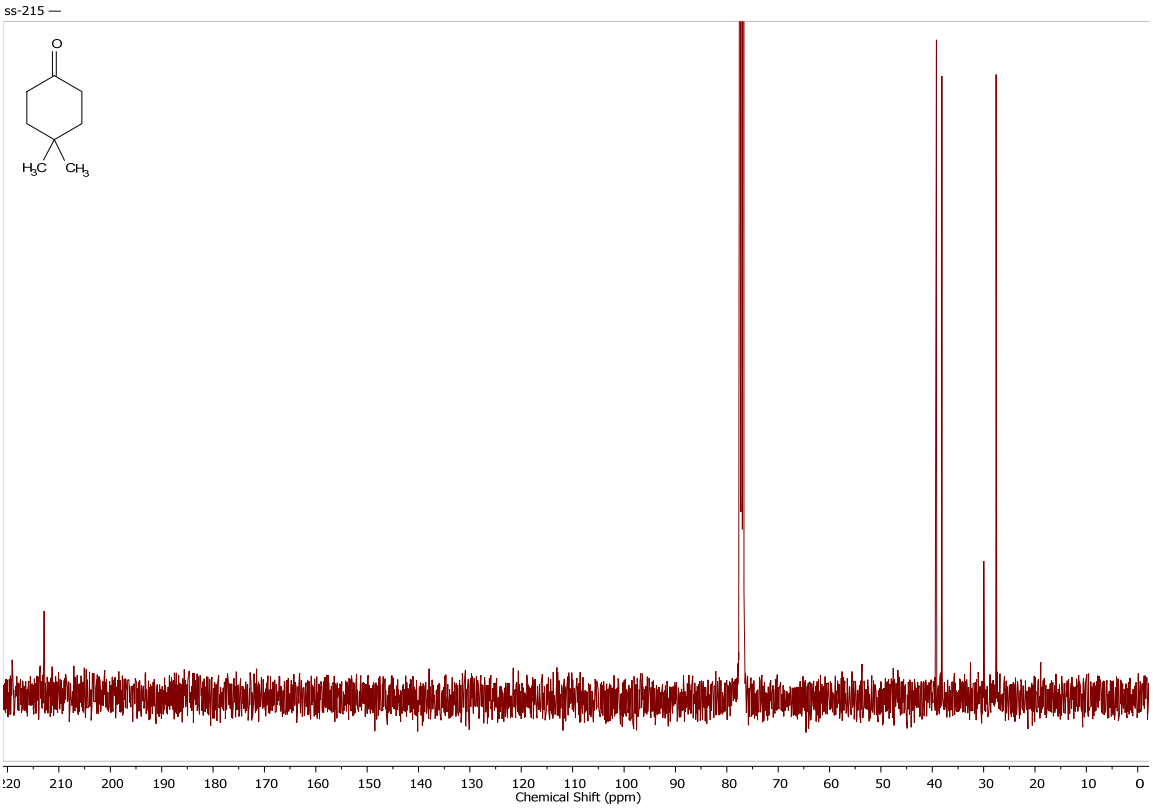


18

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.33 (t, *J* = 6.9 Hz, 4H), 1.66 (t, *J* = 6.9 Hz, 4H), 1.08 (s, 6H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 212.9, 39.2 (s, 2C) 38.1 (s, 2C) 29.6 - 30.7 (m, 1C) 27.6 (s, 2C) ppm.


ss-215 —
O
H3C
CH3
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Chemical Shift (ppm)



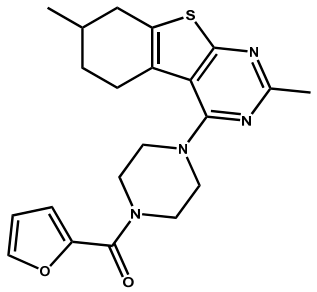
3.10 Synthesis and Characterization of Analogs[12]

The syntheses of all analogs were completed under the same reaction condition seen below.

Synthesis of (4-(2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-yl)piperazin-1-yl)(furan-2-yl)methanone (Analog 2).[12]

To a dry 10 mL round bottom flask was added chloropyrimidine (**14**) (0.70 g, 0.278 mmol), furoyl piperazine (**10**) (0.10 g, 0.555 mmol), triethylamine (0.12 mL, 0.825 mmol), and dry methanol (5.0 mL, 0.089 M). The reaction was then left to react for 24 hours at room temperature. Chromatography (20:80 mixture of ethyl acetate: hexane) is

then completed in order to obtain analog **2** (0.079 g, 0.199 mmol), at a 64% yield of a tan solid.

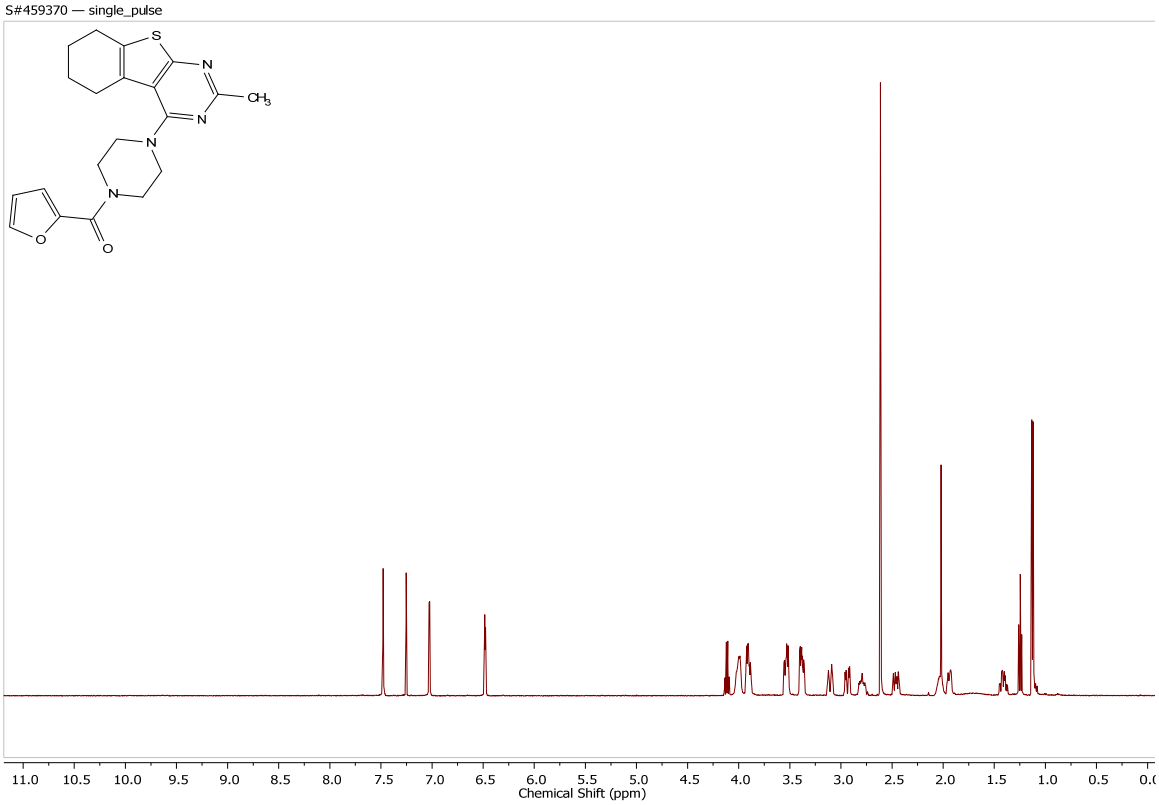


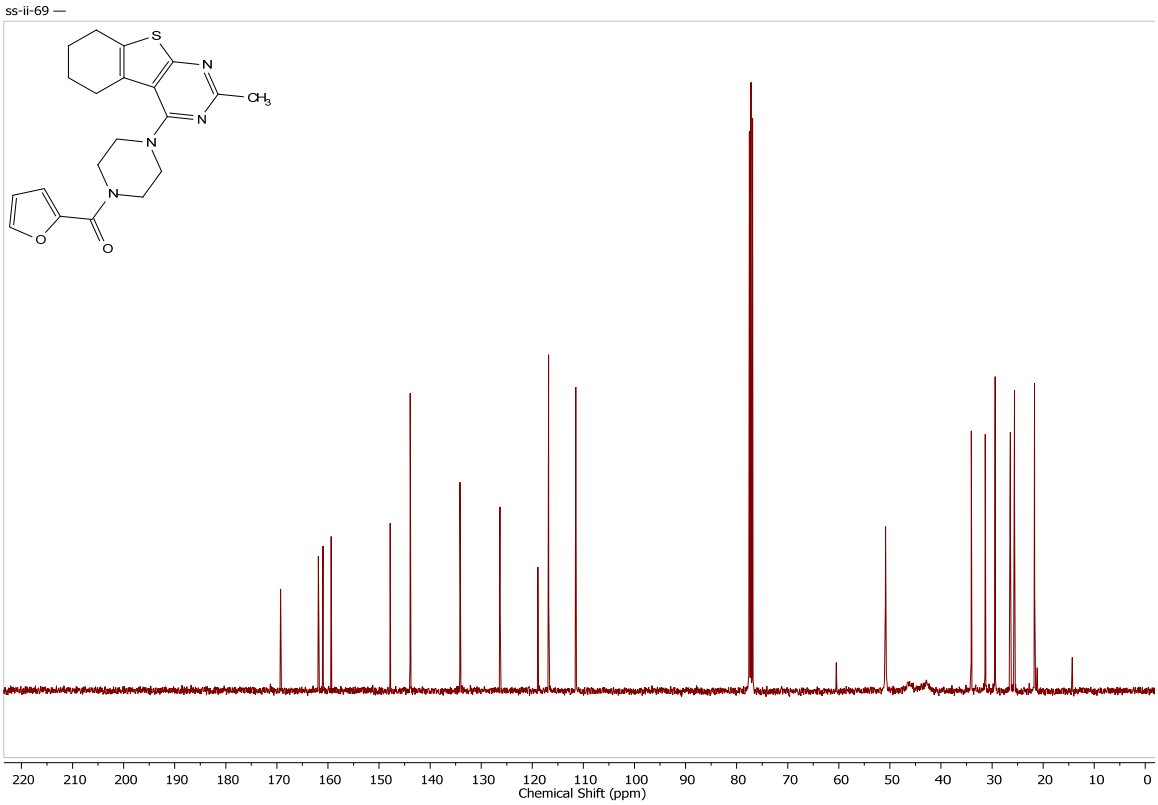
Analog 2

^{1}H NMR (500 MHz, $CDCl_3$, 58 °C**) δ** 7.48 (s, 1H), 7.03 (d, *J* = 3.4 Hz, 1H), 6.48 (dd, *J* = 1.7, 3.4 Hz, 1H), 4.06 - 3.96 (m, 2H), 3.95 - 3.86 (m, 2H), 3.53 (ddd, *J* = 3.2, 7.2, 12.6 Hz, 2H), 3.38 (ddd, *J* = 2.9, 7.2, 12.9 Hz, 2H), 3.11 (d, *J* = 16.0 Hz, 1H), 2.94 (dd, *J* = 5.4, 16.9 Hz, 1H), 2.80 (ddt, J_d = 2.6, 5.2 Hz J_t = 10.7 Hz, 1H), 2.61 (s, 3H), 2.51 - 2.41 (m, 1H), 2.08 - 1.98 (m, 1H), 1.97 - 1.90 (m, 1H), 1.48 - 1.35 (m, 1H), 1.13 (d, *J* = 6.3 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 161.9, 161.0, 159.4, 147.8, 143.9, 134.2, 126.3, 118.9, 116.8, 111.5, 60.5, 50.8, 44.6, 34.0, 31.3, 29.4, 26.4, 25.6, 21.7, 14.3 ppm.

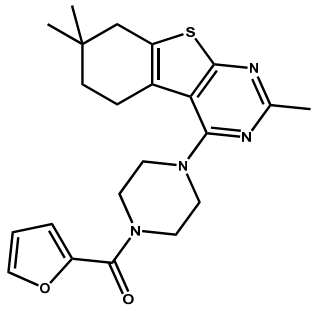
FTMS ESI: $C_{21}H_{25}N_4O_2S$ $[M+H]^+$, Calculated: 397.1698 g/mol, Found: 397.1679 g/mol.


S#459370 — single_pulse
S
N
N
CH3
N
N
O
O
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Chemical Shift (ppm)



Synthesis of furan-2-yl(4-(2,7,7-trimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)piperazin-1-yl)methanone (Analog 3).[12]

To a dry 5 mL round bottom flask was added chloropyrimidine (**21**) (0.038 g, 0.143 mmol), furoyl piperazine (**10**) (0.0515 g, 0.0515 mmol), triethylamine (0.06 mL, 0.429 mmol), and dry methanol (2.6 mL, 0.055 M). The reaction was then left to react for 24 hours at room temperature. Chromatography (20:80 mixture of ethyl acetate: hexane) is then completed in order to obtain analog **3** (0.044 g, 0.107 mmol), at a 75% yield of a tan solid.

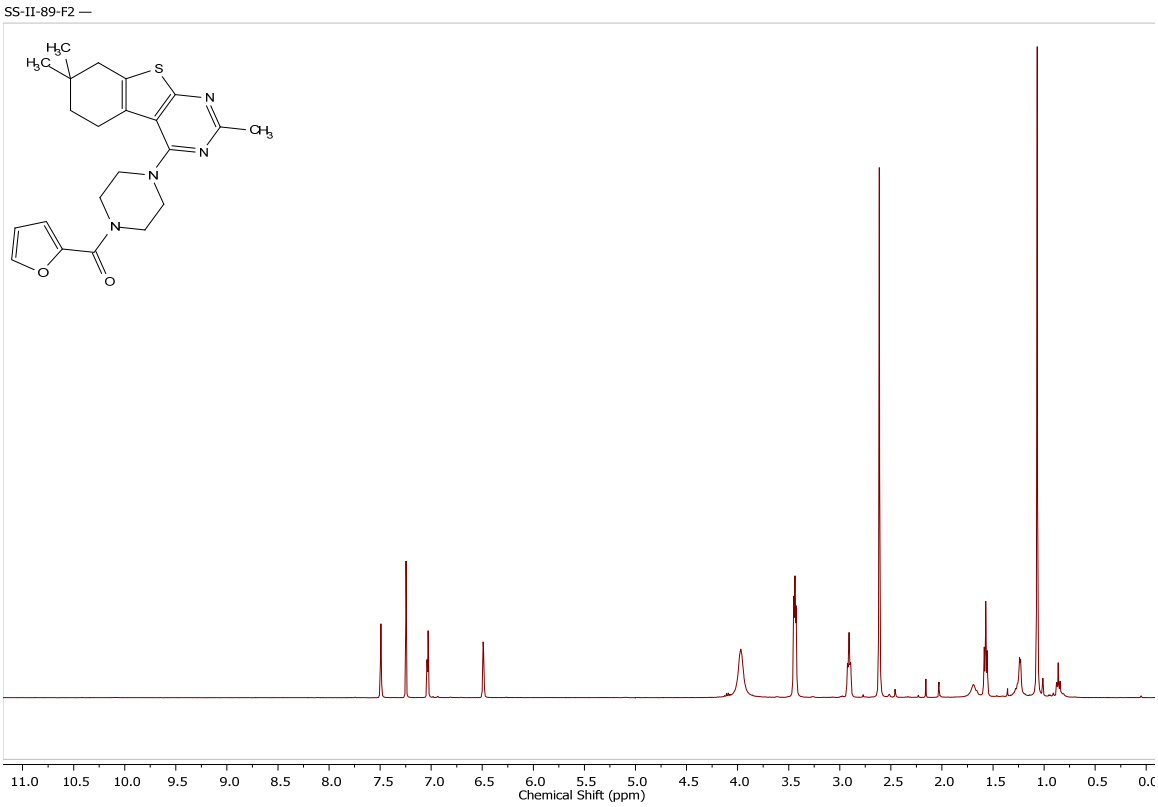


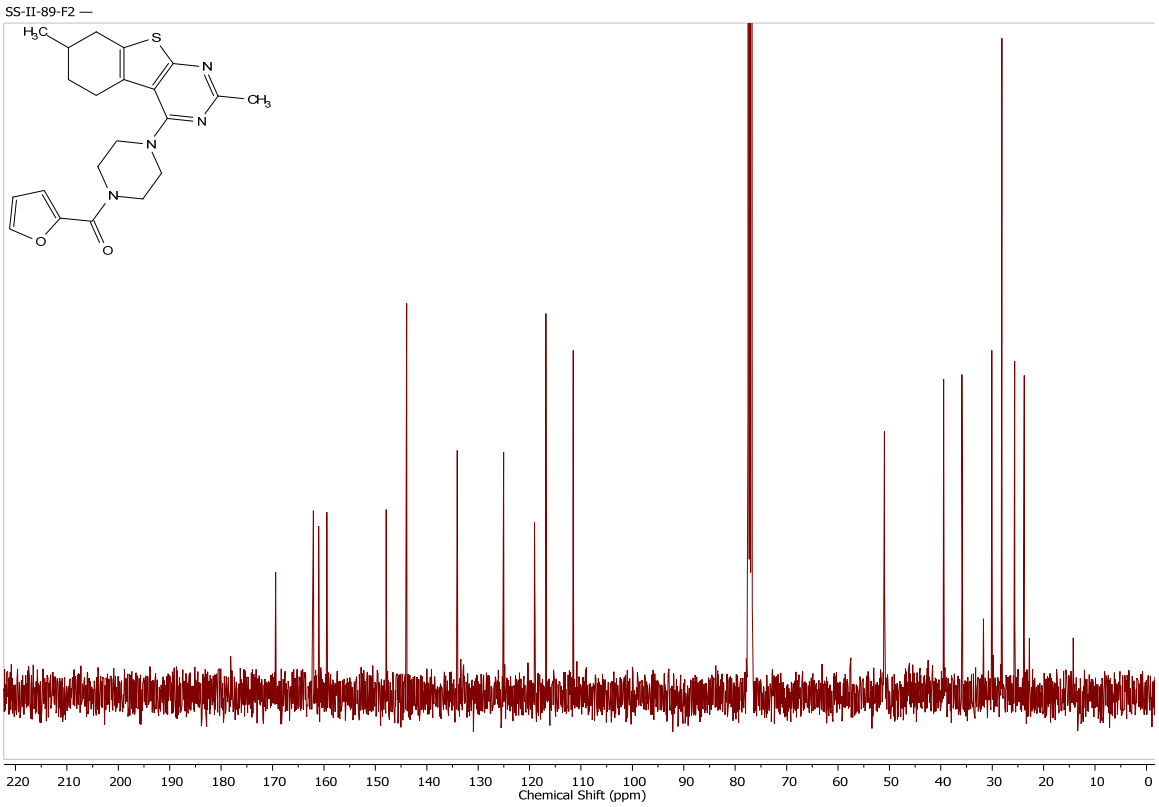
Analog 3

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (s, 1H), 7.03 (d, *J* = 3.2 Hz, 1H), 6.49 (dd, *J* = 1.8, 3.2 Hz, 1H), 3.97 (br. s, 4H), 3.52 - 3.24 (m, 4H), 2.91 (t, *J* = 6.2 Hz, 2H), 2.61 (s, 3H), 1.57 (t, *J* = 6.2 Hz, 2H), 1.35 - 1.16 (m, 2H), 1.13 - 0.93 (m, 6H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 162.1, 161.0, 159.4, 147.9, 143.9, 134.1, 125.1, 119.0, 116.8, 111.5, 51.0, 39.5, 35.9, 31.7, 30.1, 28.1 (2C), 25.6, 23.8, 22.8, 14.3 ppm.

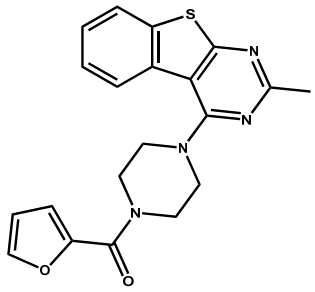
FTMS ESI: $C_{22}H_{27}N_4O_2S$ $[M+H]^+$, Calculated: 411.18492 g/mol Found: 411.18497 g/mol.


SS-II-89-F2 —
Chemical Shift (ppm)



Synthesis of furan-2-yl(4-(2-methylbenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)piperazin-1-yl)methanone (Analog 4).[12]

To a dry 0.5-2.0 mL microwave vial was added chloropyrimidine (**28**) (0.012 g, 0.053 mmol), furoyl piperazine (**10**) (0.019 g, 0.106 mmol), triethylamine (0.02 mL, 0.159 mmol), and dry methanol (1.0 mL, 0.053 M). The reaction was then left to react for 24 hours at room temperature then 24 hours at 50 °C. Chromatography (hexane) is then completed in order to obtain analog **3** (0.0036 g, 0.0095 mmol), at an 18% yield of a brown solid.

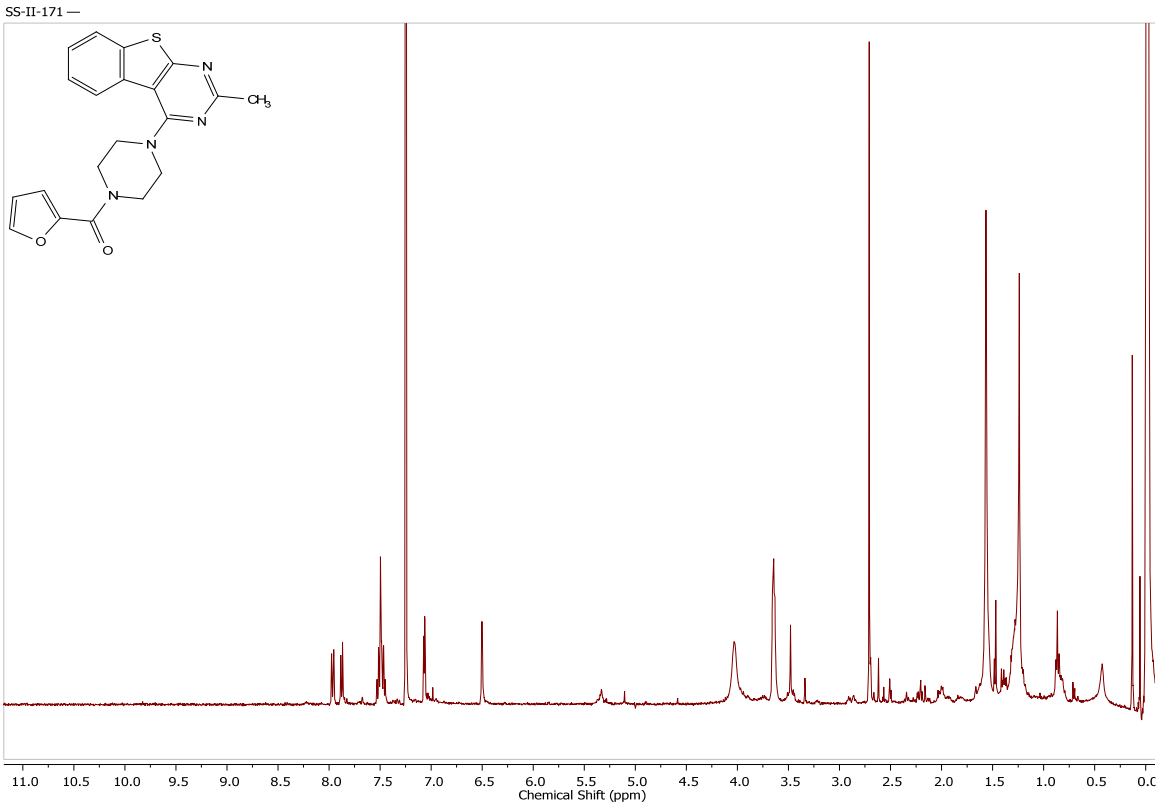


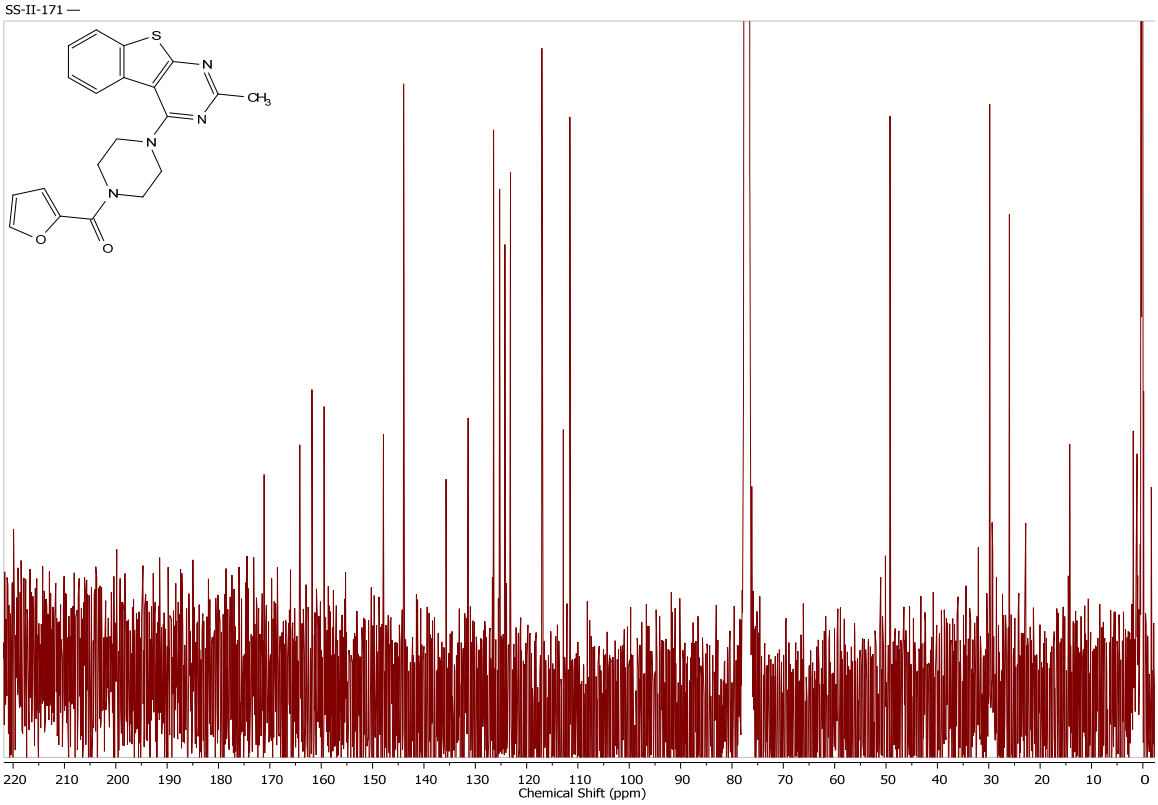
Analog 4

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 - 7.93 (m, 1H), 7.91 - 7.84 (m, 1H), 7.55 - 7.43 (m, 3H), 7.10 - 7.03 (m, 1H), 6.53 - 6.47 (m, 1H), 4.11 - 3.95 (m, 4H), 3.69 - 3.58 (m, 4H), 2.73 - 2.70 (m, 3H) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 164.8, 161.7, 158.9, 148.0, 143.7, 135.3, 131.2, 126.5, 125.2, 124.2, 123.1, 116.8, 113.0, 111.2, 48.9, 29.8, 25.8 ppm

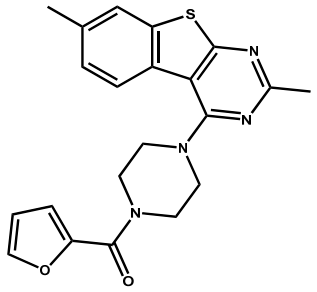
FTMS ESI: $C_{20}H_{18}N_4O_2S$ $[M+H]^+$, Calculated: 379.1228 Found: 379.1216


SS-II-171
S
N
N
CH3
N
N
O
O
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Chemical Shift (ppm)



Synthesis of (4-(2,7-dimethylbenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)piperazin-1-yl)(furan-2-yl)methanone (Analog 5).[12]

To a dry 5.0-20.0 mL microwave vial was added chloropyrimidine (**29**) (0.035 g, 0.141 mmol), furoyl piperazine (**10**) (0.051 g, 0.282 mmol), triethylamine (0.03 mL, 0.423 mmol), and dry methanol (2.6 mL, 0.055 M). The reaction was then left to react for 24 hours at room temperature then 24 hours at 50 °C. Chromatography (hexane) is then completed in order to obtain analog **3** (0.0054 g, 0.014 mmol), at a 10% yield of a brown solid.

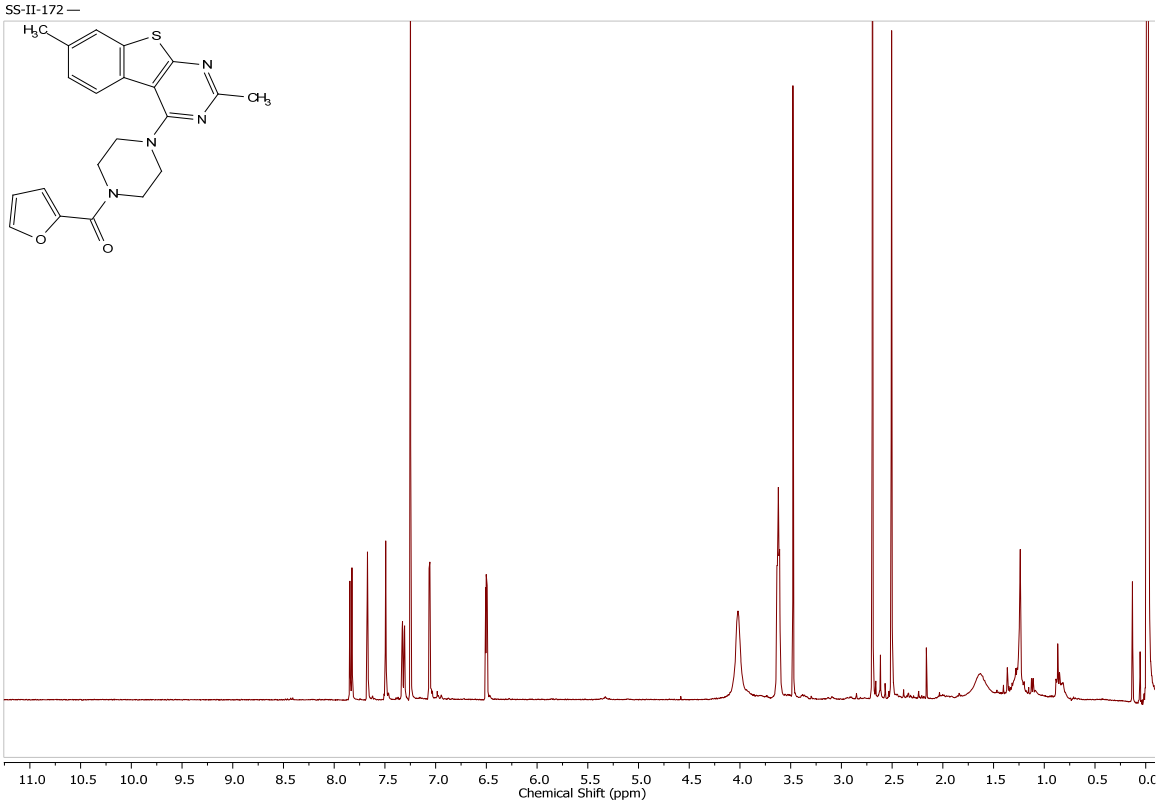


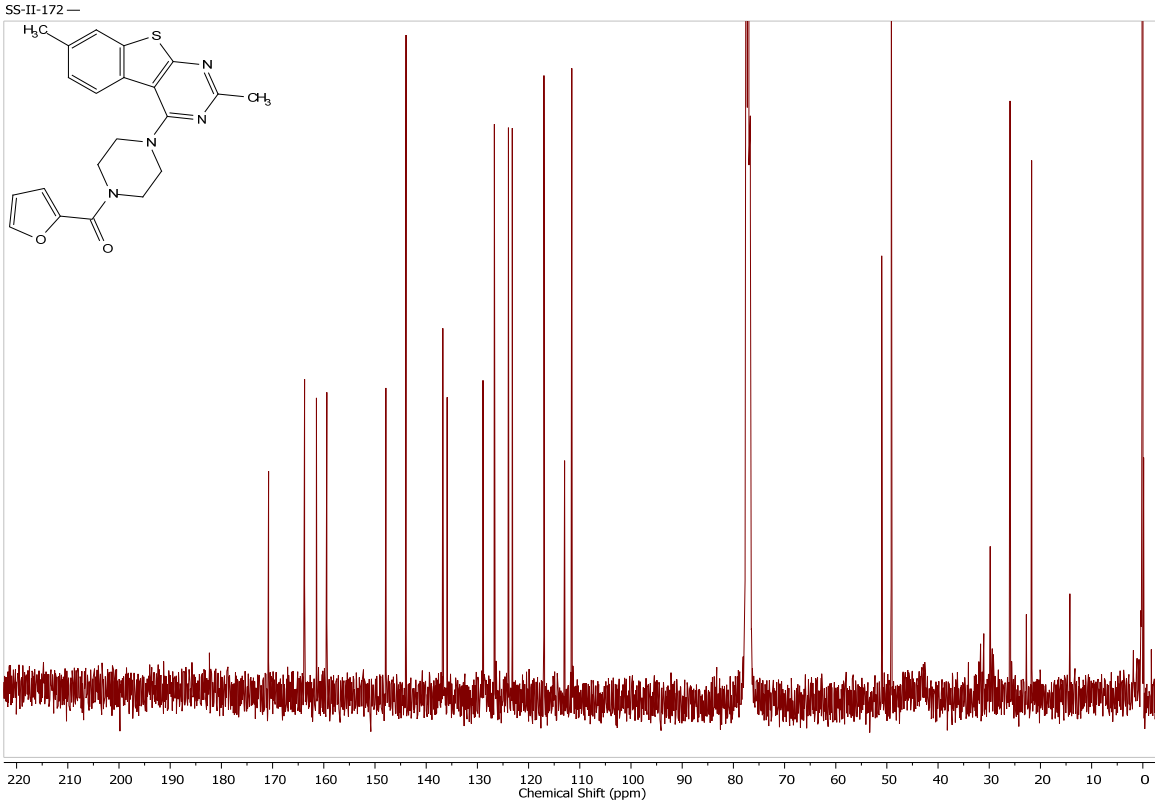
Analog 5

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 - 7.79 (m, 1H), 7.69 - 7.64 (m, 1H), 7.52 - 7.43 (m, 1H), 7.36 - 7.30 (m, 1H), 7.12 - 7.03 (m, 1H), 6.53 - 6.47 (m, 1H), 4.08 - 3.93 (m, 4H), 3.68 - 3.52 (m, 4H), 2.69 (s, 3H), 2.51 (s, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 163.7, 161.6, 159.2, 147.3, 143.9, 136.8, 135.6, 129.2, 126.7, 124.1, 123.1, 117.0, 113.2, 111.6, 51.0, 49.0 (2C), 25.9 (2C), 21.3 ppm.

FTMS ESI: $C_{22}H_{21}N_4O_2S$ $[M+H]^+$, Calculated: 393.1385 g/mol, Found: 393.1371 g/mol.


SS-II-172
H3C
S
N
CH3
N
N
N
O
O
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
Chemical Shift (ppm)


SS-II-172
H3C
S
N
CH3
N
N
N
O
O
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
Chemical Shift (ppm)

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