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Synthesis of 2,4-Disubstituted Pyrimidine Derivatives as Potential 5-HT7 Receptor Antagonist.

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SYNTHESIS OF 2,4-DISUBSTITUTED PYRIMIDINE DERIVATIVES AS POTENTIAL

5-HT$_7$ RECEPTOR ANTAGONIST.

by

Shannon M. Sullivan

Under the direction of Dr. Lucjan Strekowski

ABSTRACT

The synthesis of a series of 2-chloropyrimidine derivatives is described. The synthesis began with a nucleophilic addition of lithiated heterocyclic molecules to the 4 position of 2-chloropyrimidine to give dihydropyrimidine intermediates. The intermediates were oxidized to the pyrimidine ring using the DDQ method. This was followed by an addition-elimination reaction of an amine to the 2-chloropyrimidine derivative. The structure and properties of the final compounds were analyzed by melting point, combustion analysis, and ^{13}C-NMR and ^{1}H-NMR spectroscopy. Biological activities *in vitro* of the synthesized compounds as antagonists of the 5-HT$_{2a}$ and 5-HT$_7$ receptors were determined by an independent laboratory.

INDEX WORDS: 2-Chloropyrimidine, Nucleophilic addition reaction, Lithiated heterocyclic organic molecules, Dihydropyrimidine, Addition-Elimination reaction, Heteroaryl, Pyrimidine, 5-HT$_{2a}$, 5-HT$_7$ receptor, 2,4-Disubstituted pyrimidines.

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Georgia State University
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DEDICATION

To my niece, Baylee Elizabeth Smith, you are the reason I chose to pursue a Chemistry degree. The best decision I have made thus far. I love you.

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To Dr. Lucjan Strekowski, professor of organic chemistry at Georgia State University, thank you for being such a wonderful advisor. It definitely has been an honor and a privilege working under your direction. You allowed me to work independently and forced me to step out of my comfort zone and apply my own knowledge of chemistry to further my skills. I have learned so much from you.

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To my lab group, thank you for all of our continual discussions about chemistry and non-chemistry related topics. All the time spent in and out of the lab, there is definitely a place in my heart that is so grateful. I could not have done it without your help. It has been a pleasure.

I would also like to acknowledge and extend my heartfelt gratitude to all the chemistry professors who taught me through out my journey at Georgia State University. Dr. A.L. Baumstark, including yourself, you lead such an inspiring group of professors.

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5-HT$_7$	5-Hydroxytryptamine receptor
THF	Tetrahydrofuran
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
NMR	Nuclear magnetic resonance spectroscopy

Chapter 1

Introduction

There are 14 identified subtype serotonin receptors which have been grouped into 7 classes, (5-HT$_{1-7}$).[1-3] The most recently identified serotonin receptor is the 5-HT$_7$ receptor.[1-4] It has been identified in humans, rats, mouse, porcine and guinea pig.[1-4] It is found in the central and peripheral nervous systems. The receptor is produced by a small group of neurons which are located in the brain stem and are able to send ascending and descending signals to a large part of the central nervous system.[1-6] The functional roles of the receptor include thermoregulation, circadian rhythm, learning and memory, signaling in the hippocampus, sleep, smooth muscle relaxation of cerebral arteries and endocrine regulation.[1-6,8] Preliminary studies on non-selective ligands have shown that the receptor may also play a functional role in mood regulation. Therefore, it is a potential target for novel drug development in the treatment of depression.[1-3] The receptor, along with all other 5-HT receptors, has a high affinity for serotonin along with other similarities, causing much difficulty in the identification of selective ligands. The ligands which are produced for this specific receptor should bind with high affinity and cause regulation of the specified 5-HT$_7$ receptor.
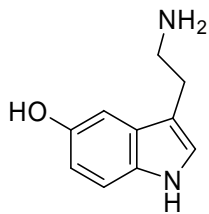

Figure 1. 5-hydroxytryptamine (serotonin, 5-HT).

The discovery of the 5-HT$_7$ receptor was made concurrently by three different research groups in 1993; Ruat *et* al, Lovenberg *et al,* and Bard *et* al.[1-3] It was found to be a seven-transmembrane domain G-protein (G$_s$) coupled receptor (GPCRs). It is embedded within the cell membrane and positively coupled to the activation of adenylate cyclase.[1-4,6-8] Distribution of the 5-HT$_7$ receptor has been studied by using autoradiography with [^{3}H]5-CT as a radioligand, *in situ* low stringency hybridization, northern-blot analysis and reverse transcriptase polymerase chain reactions (RT-PCR).[1,6]
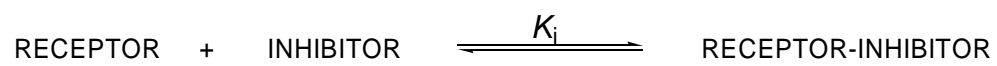
Table 1. Amino acid change lengths of 5-HT$_7$ splice variants.[1,4-5]

	5-HT$_{(7a)}$	5-HT$_{(7b)}$	5-HT$_{(7c)}$	5-HT$_{(7d)}$
Human	445	432	-	479
Rat	448	435	470	-

As shown in Table 1, four splice variants have been identified for the 5-HT$_7$ receptor in human and rat tissue; 5-HT$_{(7a)}$, 5-HT$_{(7b)}$, 5-HT$_{(7c)}$ and 5-HT$_{(7d)}$. They vary in structure and length of their carboxy terminal chain due to alternative splicing but there is little difference between their function.[1,4-5] Each of the species only expresses three of the four splice variants; two isoforms are homologous (5-HT$_{(7a)}$, 5-HT$_{(7b)}$) and humans express 5-HT$_{(7d)}$.[4]

There are many similarities of binding affinities between the 5-HT$_7$ receptor and the 5-HT$_{(1)}$ and 5-HT$_{(2a)}$ receptors. What once was thought of as a selective antagonist towards HT$_{(1)}$ and 5-HT$_{(2a)}$ receptors now shows moderate to high binding affinity towards the 5-HT$_7$ receptor. The development of selective 5-HT$_7$ receptor agonists and antagonists is crucial to help further

the knowledge of the physiological and pharmacological roles of this specific receptor. Studies performed by M. Kolaczkowski *et al* used molecular modeling techniques to better understand the binding of the 5-HT$_7$ receptor. The group used a flexible docking technique and 5-HT$_{(1a)}$ as a reference for the study due to similarities in affinity towards many ligands.[8] Using 31 different known 5-HT$_7$ receptor antagonists, two receptor-based pharmacophores were generated. The characteristics for high affinity binding towards the 5-HT$_7$ receptor and the specific interactions with the binding pockets were proposed.[8] The binding sites were found to be located along the transmembrane helix (TMH) with a central amino acid, asparagine, which acts as the main anchoring point for ligands.[8] Two binding pockets were identified; TMH 4-6, buried within the receptor and TMH 7-3, located on the extracellular side. The important features identified for high affinity binding towards the 5-HT$_7$ receptor include the interaction of a protonated nitrogen and an aromatic ring in the TMH 7-3 pocket, hydrogen bond acceptor or a hydrophobic aromatic group interacting with the TMH 4-6 pocket, and the ability of a terminal aromatic imide or amide for π-π stacking with phenylalanine and/or ion-π interactions with arganine.[8] Among the 31 different antagonists studied, the arylsulfonamidoalkylamides, shown in Figure 3, possess the most optimal geometry towards the 5-HT$_7$ receptor. The aromatic group allows for π-π stacking with phenylalanine, the nitrogen possesses ion-π interactions with arganine, and the oxygen on sulfur can hydrogen bond with tyrosine. This general template has aided in the design of novel analogues of the pyrimidine based 5-HT$_7$ ligands. The affinity towards the receptor is quantified by the study of the equilibrium between the receptor-inhibitor complex (Figure 2). The value reported as K_i is an equilibrium inhibitor constant indicating the ratio between the receptor-inhibitor complex against the free receptors. The inhibitory effect is considered to increase with decreasing K_i values.[9]

RECEPTOR   +   INHIBITOR $\xrightleftharpoons{K_i}$ RECEPTOR-INHIBITOR

$$K_i \quad = \quad \frac{[R]\,[I]}{[RI]}$$

$$pK_i \quad = \quad -\log K_i$$

Figure 2. The inhibitor constant, K_i.[9]

SB-656104 pKi = 8.7

SB-258741 pKi = 8.5

SB-269970 pKi = 8.9

SB-258719 pKi = 7.5
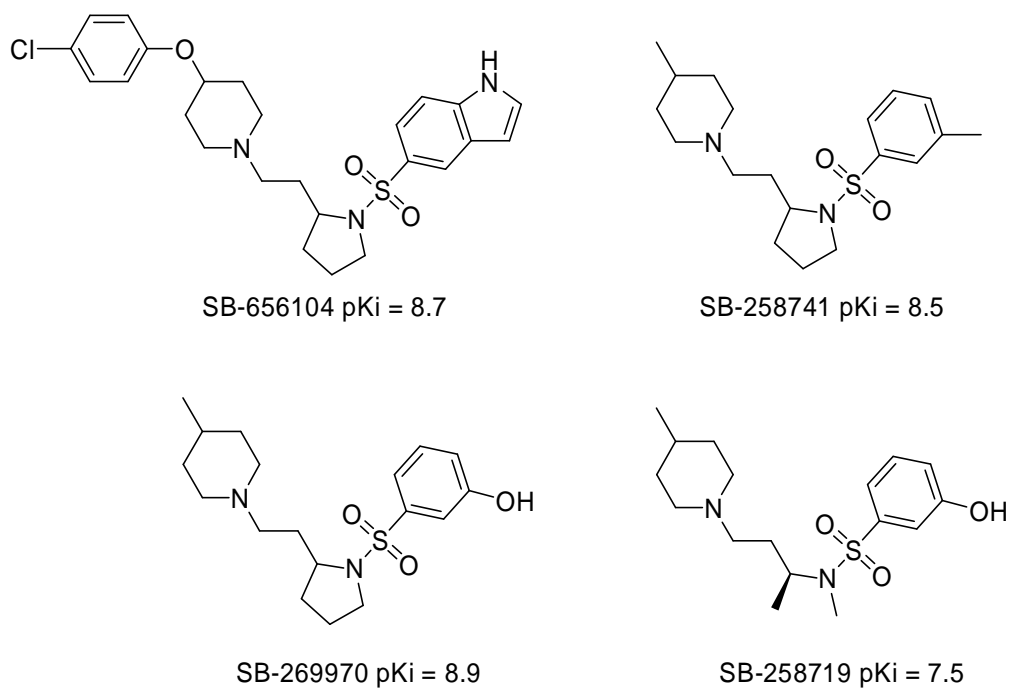
Figure 3. Arylsulfonamidoalkylamides used for binding study against the 5-HT$_7$ receptor.[8]

5-CT pKi = 9.0 8-OH DPAT pKi = 6.3 Methiothepin pKi = 8.4
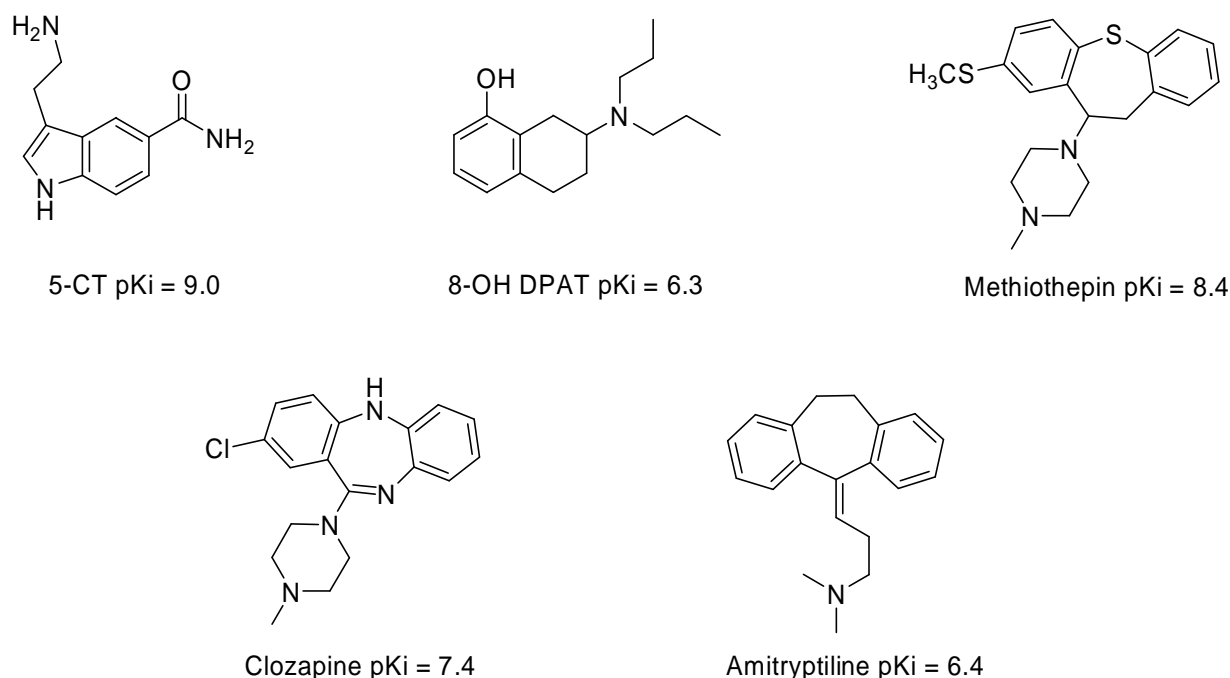
Clozapine pKi = 7.4 Amitryptiline pKi = 6.4

Figure 4. Non-selective ligands towards the 5-HT receptor.[2]

Most of what is known about the 5-HT_7 receptor comes from the use of non-selective agonists and antagonists such as 5-carboxytryptamine (5-CT), 8-OH DPAT, methiopthepin, clozapine, and amitryptyline that are also selective $5\text{-HT}_{(1a/1d)}$ receptor agonist (Figure 4).[2] An agonist binds to a specific receptor and triggers a response in the cell which mimics the action of a hormone or neurotransmitter that binds to the same receptor. An antagonist will bind to a specific receptor, blocking the agonist from binding, and will not produce a response. Such non-selective agonists and antagonists represent a class of antipsychotic and antidepressant drugs which have high or modest affinity for the 5-HT_7 receptor (Table 2). Studies have shown that use of these drugs activates the release of the early *Fos* gene in to the suprachiasmatic nuclei (SCN) of the hypothalamus. Persistent use of these drugs showed a significant decrease in 5-HT_7 binding sites in the hypothalamus corresponding to a down-regulation of the 5-HT_7 receptor.[10]

Table 2. Binding affinities for non-selective antipsychotic and antidepressant drugs towards the 5-HT$_7$ receptor.[2]

	pK$_i$		
	Rat	**Human**	**Guinea Pig**
5-HT	8.8	8.1	9.0
5-CT	9.8	9	9.4
5-MeOT	9.2	8.3	9.0
8-OH DPAT	7.5	6.3	7.3
Methiothepin	9.4	8.4	8.0
Clozapine	7.4	-	-
Amitriptyline	7.0	-	-

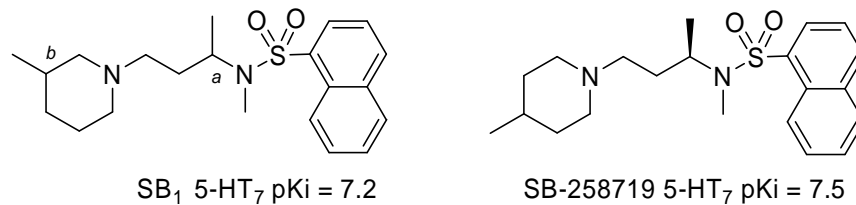SB$_1$ 5-HT$_7$ pKi = 7.2   SB-258719 5-HT$_7$ pKi = 7.5

Figure 5. SmithKline Beechman selective antagonists towards the 5-HT$_7$ receptor.[2]

In 1994, a pharmaceutical company SmithKline Beechman patented a sulfonamide compound that had an affinity (pK_i = 7.2) for the 5-HT$_7$ receptor (Figure 5). Used as a reference compound, structural modifications were performed to SB$_1$ by altering the chirality about the a and b carbons and the Structure-Activity Relationship (SAR) of the compounds was studied. Binding affinity studies showed that the R chirality on carbon a was necessary for high affinity to the 5-HT$_7$ receptor. Alteration to the b chiral center by moving the methyl group to position 4 of the piperidine ring gives sulfonamide compound SB258719. Binding affinity studies showed SB258719 to have greater than 100-fold selectivity towards the 5-HT$_7$ receptor. With a relatively high affinity, pK_i = 7.5, it showed for the first promising selective ligand.[2,4,7]
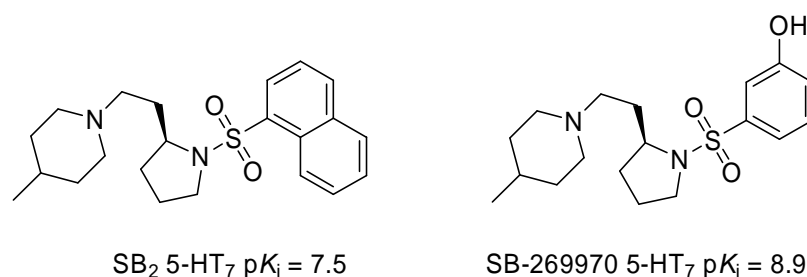
Figure 6. SB269970; selective antagonist for the 5-HT$_7$ receptor.[4]

Further structural modifications and restraints to the selective antagonist SB258719 led to the discovery of another selective antagonistic compound SB269970 (Figure 6). The freely rotating side chain was incorporated into a piperidine or pyrrolidine substituent.[4] The chiral center present on the piperidine ring in both SB$_2$ and SB269970 showed importance for affinity to the 5-HT$_7$ receptor and were used further in conformational analysis. Binding affinity studies showed that SB269970 had a high affinity for the 5-HT$_7$ receptor with a pK_i = 8.9 and has greater than 25 fold selectivity over other 5-HT receptors; however it had some binding affinity to the 5-HT$_{5A}$ receptor, pK_i ≈ 7.2.[4] SB269970 was further tested for the ability to stimulate adenylate cyclase in HEK 293 cells and showed antagonistic properties against the 5-HT$_7$ receptor. Although SB269970 showed great selectivity and inhibition, it showed for a poor drug candidate. This was due to its short life span *in vivo* caused by the phenol group.[4] Researchers then decided to replace the phenol group with a variety of arenes to test for selectivity and bioavailability.
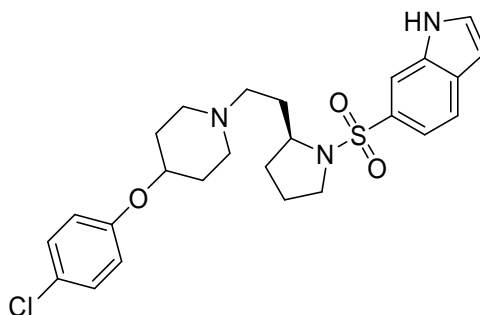
SB-656104 5-HT$_7$ pK_i = 8.7

Figure 7. GlaxoSmithKline compound SB-656104.[2]

GlaxoSmithKline have developed more metabolically stable compounds which resemble SB269970. The phenol group of SB269970 was replaced with arene groups to study the binding affinities against the 5-HT$_7$ receptor. All the compounds showed a significant loss in binding affinity; a compound with a 6-methylindole group is the only one which came close to the SB269970 affinity (pK_i = 8.9).[2] GlaxoSmithKline then decided to replace the methyl group with arene groups; indole, benzimidazolone, and flurophenoxy showed greatest increase in affinity. The results showed a significant increase in binding affinities but also affinity for the adrenergic α$_{1B}$ receptor. Due to its high affinity for 5-HT$_7$ receptor, lowered affinity for α$_{1B}$ receptor and bioavailability of 16%, SB-656104 was further studied. Compared to all the 5-HT receptors, SB-656104 (Figure 7) showed greater than a 100 fold binding selectivity over all except the 5-HT$_{1D}$, 5-HT$_{2A}$, and 5-HT$_{2B}$ receptors.[2]
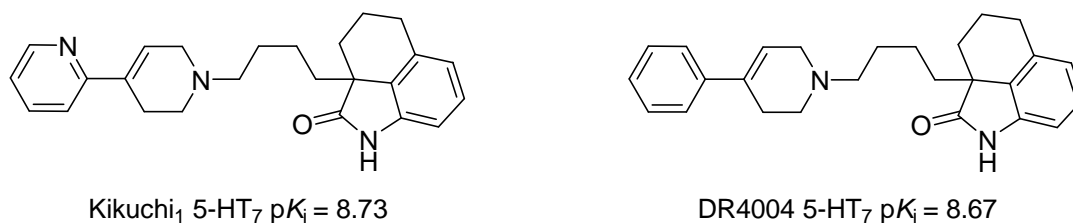
Kikuchi$_1$ 5-HT$_7$ pK_i = 8.73   DR4004 5-HT$_7$ pK_i = 8.67

Figure 8. Kikuchi compounds with high affinity to 5-HT$_7$ receptor.[2,4,7]

Another promising selective antagonist for the 5-HT$_7$ receptor was synthesized by

Kikuchi *et al* (Figure 8). A tetrahydrobenzindole derivative, DR4004, showed high affinity and

selectivity towards the 5-HT$_7$ receptor[2,4,7]. The antagonist DR4004 demonstrated a pK_i of 8.67

and compared with the 5-HT$_2$ receptor, it showed a 47-fold selectivity towards the 5-HT$_7$

receptor [2,7]. Such selectivity allowed for the determination that an aromatic group near the basic

nitrogen is required to achieve such high selectivity. DR4004 was further evaluated with the 5-

HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_4$, 5-HT$_6$, and D$_2$ receptors. It was also found to be highly selective towards
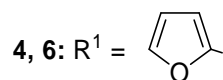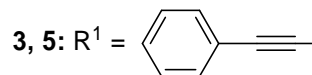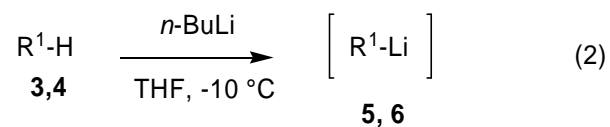
the 5-HT$_2$ receptor.

Chapter 2

Discussion

      The protocol for the synthesis of the pyrimidine derivatives was developed by Strekowski *et al* at Georgia State University.[11-13] Initial research on this project dealt with 2-, 4- and 6-halogenopyrimidines reacting with commercially available organometallic reagents. The group went further to add organolithium reagents to generate 2,4-disubstituted pyrimidines and also to 2,4,6-trisubstituted pyrimidines.

$$\text{(1)}$$
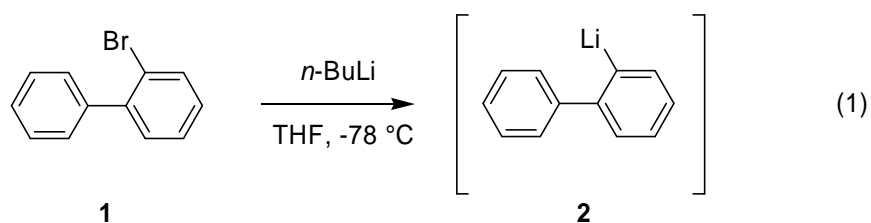
      The first step of the reaction consists of a bromine-lithium exchange reaction (Equation 1) or a lithiation (Equation 2) of an organic molecule to generate the desired organolithium reagent. Bromine lithium exchange was conducted in anhydrous THF at low temperatures under static pressure of nitrogen. An excess of substrate **1** was used to ensure complete consumption of *n*-butyllithium. The commercially available *n*-butyllithium acts as a strong nucleophile by displacing the bromine with lithium resulting in the desired organolithium reagent.

$$\text{R}^1\text{-H} \quad \xrightarrow[\text{THF, -10 °C}]{n\text{-BuLi}} \quad \left[\, \text{R}^1\text{-Li} \,\right] \qquad (2)$$

3,4 **5, 6**

3, 5: R^1 =

4, 6: R^1 =

Lithiation of compounds **3** and **4** occurred under much milder conditions in order to cleave the bond between carbon and hydrogen. In lithiation, the *n*-butyllithium acts as a base and will deprotonate the C-H bond and reestablish the bond with lithium. The process is reversible forming the more stable organolithium reagent.

Scheme 1. Synthesis of Compounds **10-12**.
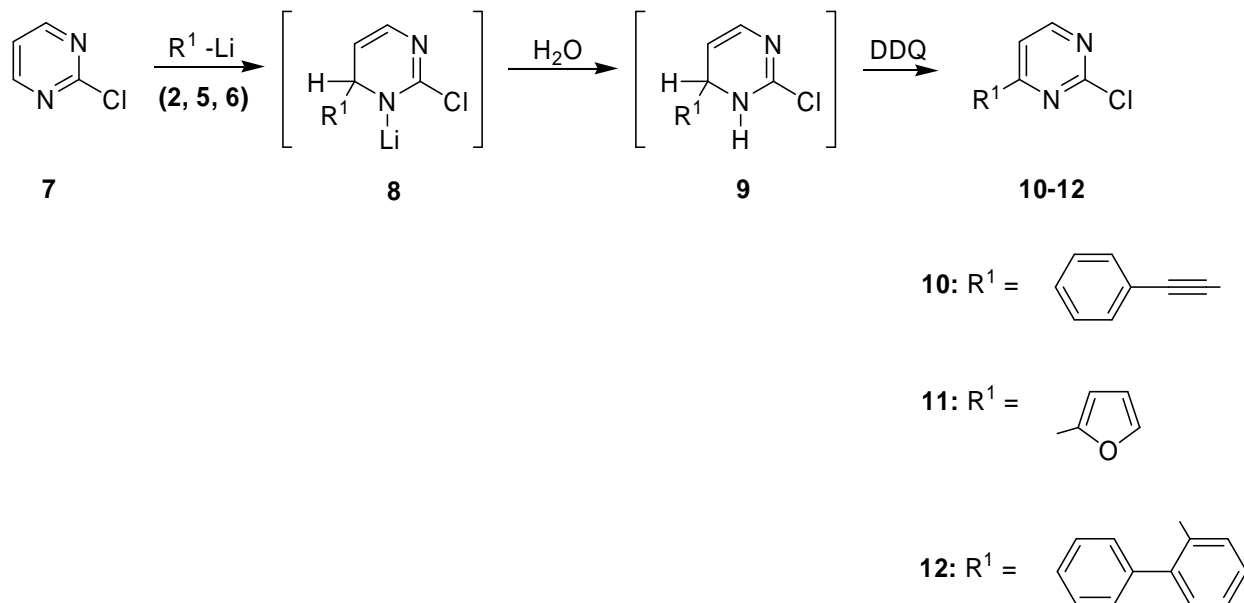
As shown in Scheme 1, 2-chloropyrimidine **(7)** was allowed to react with organolithium

reagents **2, 5,** and **6**, in anhydrous THF. The corresponding dihydropyrimidine **(9)** intermediate

was treated with 2,3- dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to reestablish the

aromaticity of the pyrimidine ring. DDQ acts as an oxidation agent by the removal of two

hydrogens and restoring the double bond in the ring. Extraction was performed using

dichloromethane. The compounds were purified on a chromatotron using normal phase silica gel

with a mobile phase of dichloromethane and ethyl acetate. The substituted pyrimidines **10-12**

were synthesized as shown in Scheme 1.

Scheme 2. Synthesis of Compound **14**.

13

14
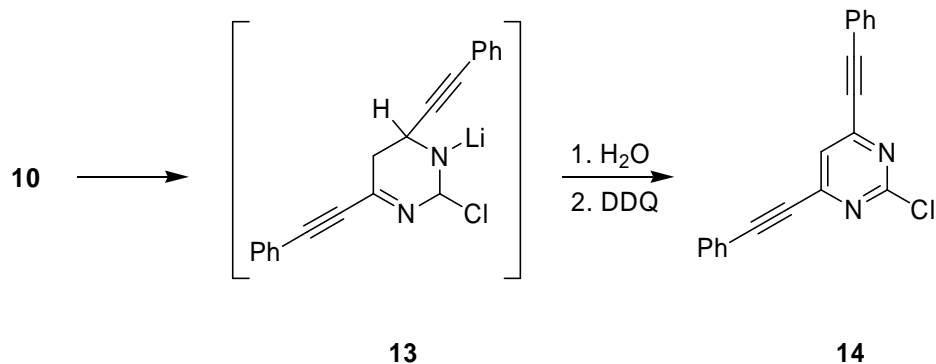
Compound **10** can be further substituted at the position 6 by a similar sequence of addition and oxidation reactions. Scheme 2 represents the second addition of organolithium reagent **5** to 2-chloro-4-(2-phenylethynyl)pyrimidine **(10)**. Quenching with water of the dihydropyrimidine intermediate **13** followed by treatment with DDQ gave 2-chloro-4,6-bis(2-phenylethynyl)pyrimidine **(14)**.

Scheme 3. Synthesis of Compounds **16-21**.

16: R^1 = ⟨phenylacetylene⟩ R^2 = –N◯N–Me **19:** R^1 = ⟨furan⟩ R^2 = –N◯NH

17: R^1 = ⟨phenylacetylene⟩ R^2 = –N◯O **20:** R^1 = ⟨furan⟩ R^2 = –N◯N–Me

18: R^1 = ⟨phenylacetylene⟩ R^2 = –N◯NH **21:** R^1 = ⟨biphenyl⟩ R^2 = –N◯N–Me
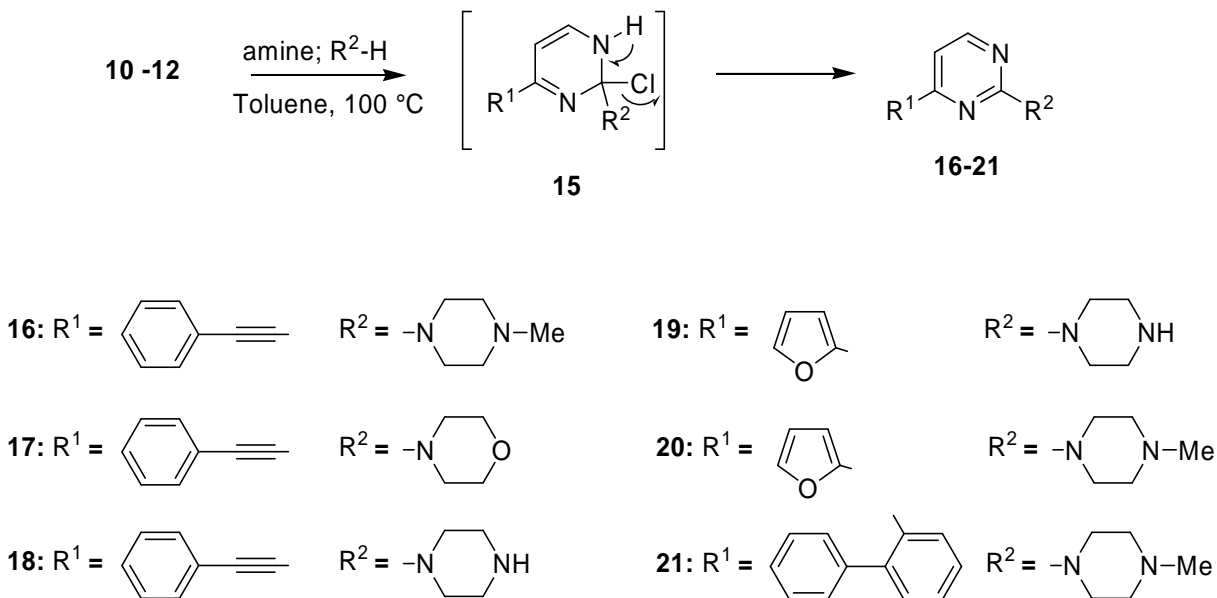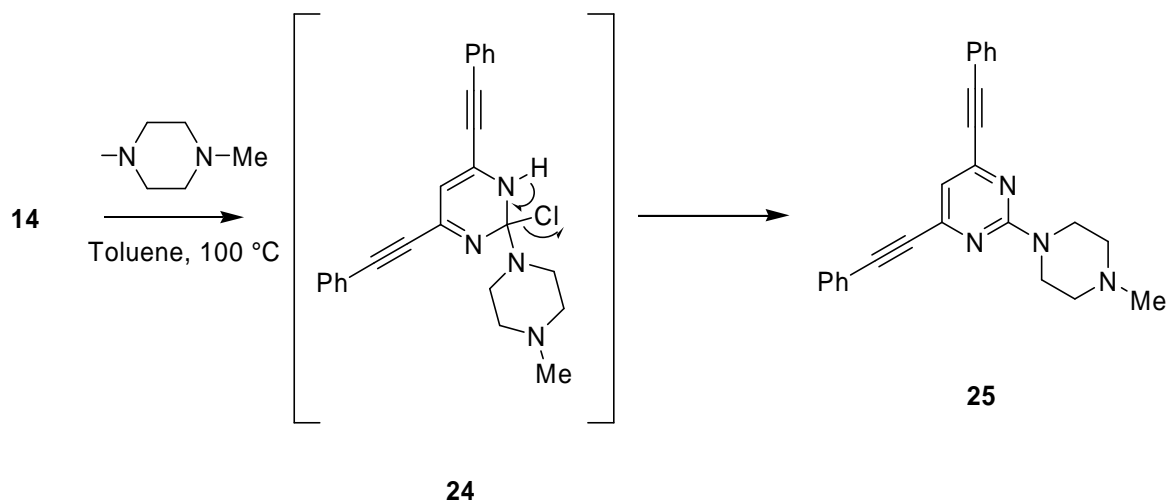
The synthesis of new pyrimidine derivatives **16-21** is shown in Scheme 3. The chloride displacement reaction was conducted in toluene under reflux for 24 h.

Scheme 4. Synthesis of Compound **23**.

22   **23**
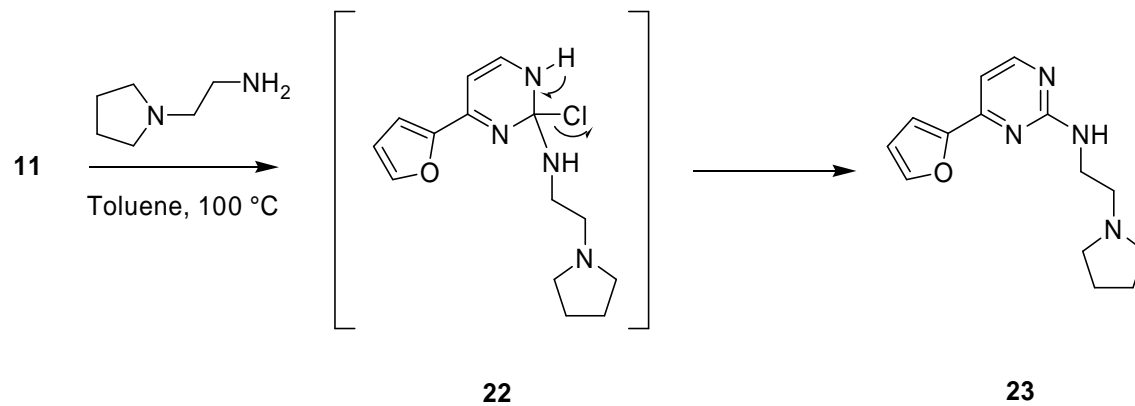
The synthesis of 4-(2-Furyl)-*N*-(2-(pyrrolidino)ethyl)pyrimidin-2-amine **(23)** through the intermediate of **22** is shown in Scheme 4.

Scheme 5. Synthesis of Compound **25**.

24

Following the same protocol, addition of excess *N*-methylpiperazine to 2-chloro-4,6-

bis(2-phenylethynyl)pyrimidine **(14)** was conducted in toluene under reflux for 24 h. Elimination

of chloride from the intermediate adduct **24** gave 2-(4-methylpiperazinyl)-4,6-bis(2-

phenylethynyl)pyrimidine **(25)** in good yield.

Scheme 6. Synthesis of Compound **28**.

26

27

28
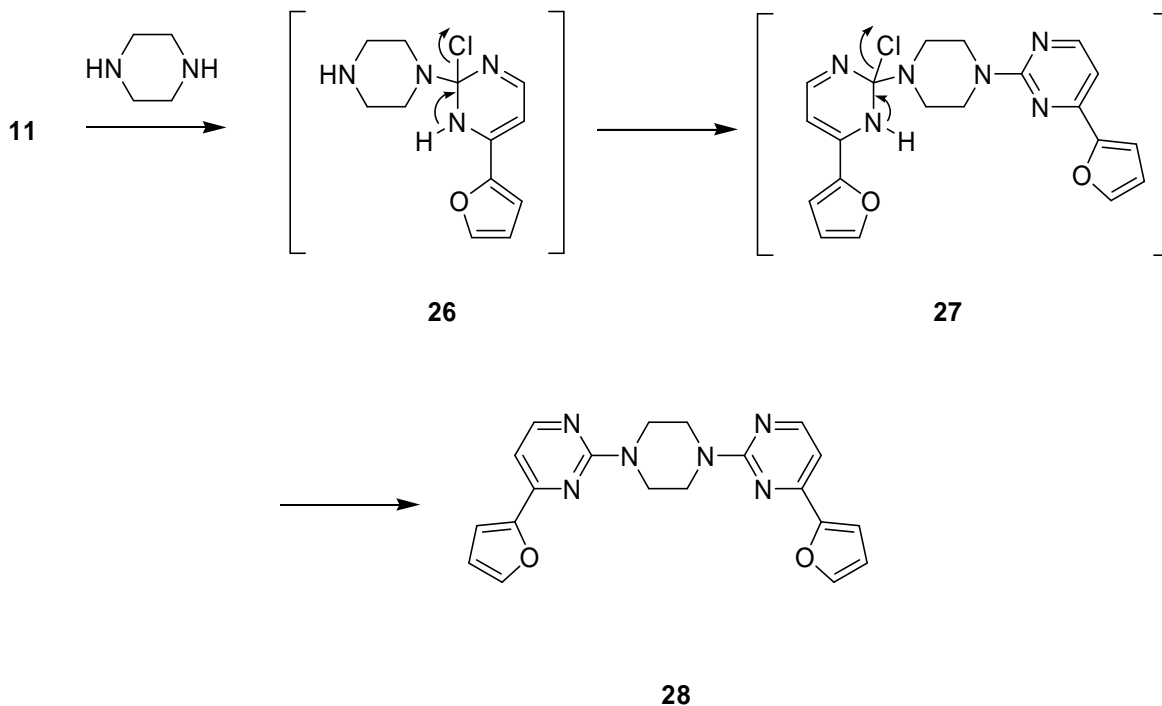
A similar synthesis of **28** is shown in Scheme 6. The reaction was conducted in toluene under reflux for 2 hrs. First piperazine attacks the electrophilic carbon on the pyrimidine ring. After displacement of the chloride, excess 2-chloro-4-(2-furyl)pyrimidine **(11)** attacks the second electrophilic carbon allowing for a second nucleophilic addition reaction to occur. The final product is 1,4-Bis[4-(furan-2-yl)pyrimidin-2-yl]piperazine **(28)**.

All substituted pyrimidines, **16-21**, **23**, **25** and **28** were evaluated by proton NMR and combustion analysis experiments.
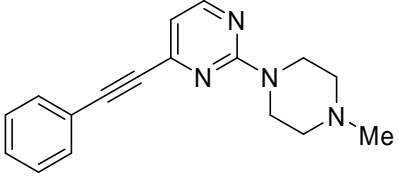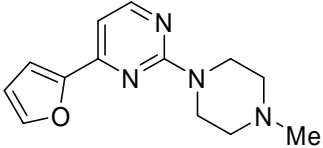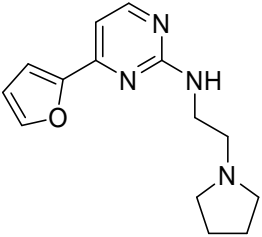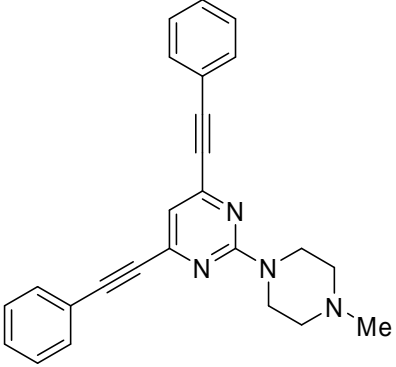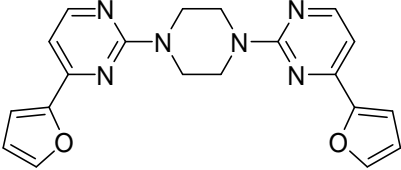
Chapter 3.

Biological Activity

The substituted pyrimidine compounds have shown high to moderate affinity towards the 5-HT$_7$ receptor along with the 5-HT$_{2A}$ receptor. The pK_i values (K_i is inhibition constant, pK_i = -log K_i) reported show inhibition towards the specific receptors. The receptors were tested using a radioligand binding assay method. The 5-HT$_{2A}$ binding assays used rat cortical membranes, [^{3}H]-ketanserin and methysergide for nonspecific binding. The 5-HT$_7$ binding assays were performed using 5-HT$_{7b}$ cloned mammalian cDNA introduced into human embryonic kidney cells (HEK 293) using [^{3}H]-CT saturation binding studies along with cAMP accumulation assays.[13]

The biological activity of the synthesized compounds **16, 20, 23, 25** and **28,** are reported in Table 3. Activity showed a moderate affinity for the 5-HT$_7$ receptor with pK_i's ranging from 5.09-6.00. The compounds showed a higher affinity towards the 5-HT$_{2A}$ receptor with pK_i's ranging from 5.13-6.25.

Table 3. Biological Activity of Ligands **16, 20, 23, 25** and **28**.

Compound		pK_i 5-HT$_{2A}$	pK_i 5-HT$_7$
16		–	5.77
20		6.25	5.99
23		5.13	5.09
25		–	6.00
28		5.76	5.27

Chapter 4.

Experimental

General

All reactions with organolithium reagents were conducted in tetrahydrofuran (THF) under static pressure of nitrogen. The glassware was cleaned and dried with heat, assembled hot and cooled in a stream of nitrogen. All liquid reagents were transferred with syringes.

Melting points were measured (open Pyrex capillary) on a Mel-Temp® and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on Varian Unity+ operating at 300 MHz and 400 MHz instrument at 25 °C using tetramethylsilane as an internal standard. NMR samples were prepared in 0.7 mL of $CDCl_3$ (Aldrich) in 5 mm NMR tubes. Chemical analysis was taken on a Perkin Elmer® Series II CHNS/O Analyser 2400 instrument.

Lithiation

Reagent **2** was generated by a bromine-lithium exchange reaction of 2-bromo-1,1-biphenylethyl with *n*-butyllithium (Aldrich, 2.5 M in hexanes, 2 mmoles) at a temperature of -78 °C for 30 min. 2-Phenylethynyllithium **(5)** and 2-furanyllithium **(6)** were generated by a lithiation reaction of phenylacetylene and furan, respectively, using *n*-butyllithium (Aldrich, 2.5 M in hexanes, 2 mmoles) at -78 °C and -10 °C for 2 hours.

Preparation of 2-chloro-4-(heteroaryl)pyrimidines:[11-13]

A solution of a heteroaryllithium reagent R^1-Li was prepared as described above. The solution was treated dropwise at -78 °C with 2-chloropyrimidine (0.23g, 2 mmoles) in THF (5 mL). The mixture was stirred for 3 hours until the temperature reached -30 °C. The progress of the reaction was monitored by TLC on silica gel, eluting with dichloromethane:hexanes (1:1), until 2-chloropyrimidine was consumed. The mixture was then quenched with deionized water (0.5 mL) and treated with a solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 1 g, 2 mmoles) in THF (5 mL). Once the mixture reached room temperature, a mixture of sodium hydroxide (3 M, 5 mL, 2 mmoles) was added and stirred. The mixture was rinsed with THF and hexanes. The organic layer was dried with anhydrous magnesium sulfate and purified by silica gel column chromatography with hexanes/dichloromethane/ethyl acetate as an eluent to give **10-12**, and **14**.

Amination

The 2-chloro-4-(heteroaryl)pyrimidines **10-14** were allowed to react with an excess amount of an amine R^2-H. The mixture was covered by septum and allowed to react for 24 h at room temperature unless specified otherwise. The reaction progress was monitored by TLC, eluting with dichloromethane:hexanes (1:1), until the 2-chloro-4-(heteroaryl)pyrimidine **10-14** was consumed.

Workup

The reaction was then quenched with deionized water (0.5 mL) and a solution of sodium carbonate (0.1 g, 2 mmoles) was added to neutralize with HCl. The mixture was then rinsed with hexanes and ether. Anhydrous magnesium sulfate was added to the organic layer for drying. The product was purified by silica gel column chromatography with hexanes/dichloromethane/ethyl acetate as an eluent to give **16**-**21, 23, 25** and **28**.

Salt Formation

Compounds **16, 17, 20-23, 25** and **28** were dissolved in minimal amount of methanol and treated with excess hydrobromic acid to form the hydrobromide salt. The salt was collected from diethyl ether using vacuum filtration to give compounds **16**, **17**, **20**, **21**, **23**, **25** and **28**, respectively. Compounds **18** and **19** hydrolyzed when trying to convert to a salt.

All salts were sent to A.J. Bojarski *et* al at the Polish Academy of Science in Krakow, Poland for biological testing.

Detailed Procedures and Characterization

2-Chloro-4-(2-phenylethynyl)pyrimidine (10)

While under N_2 pressure and a temperature of -78 °C, *n*-butyllithium (2.5 M, 1.57 mL, 3.9 mmoles) was added to phenylacetylene (0.401 g, 3.93 mmol) dissolved in THF (10 mL). The reaction mixture was held at a steady temperature of -78 °C for 30 minutes. Then, a solution of 2-chloropyrimidine **7** (0.30 g, 2.62 mmoles) in THF (3 mL) was added to the above mixture. The temperature was allowed to reach -30 °C over 3 hours. Workup according to general method described above yielded 0.15 g (0.47 mmol, 37%) of 2-chloro-4,6-bis(2-phenylethynyl)pyrimidine **(10)**.

This compound had mp 102-105 °C; ^{1}H-NMR (300 MHz, CDCl$_3$): **δ** 8.65 (d, 1H, *J* = 5.1 Hz), 7.66 (d, 1H, *J* = 3.0 Hz), 7.64 (d, 1H, *J* = 3.9 Hz), 7.46 (m, 4H). ^{13}C-NMR (300 MHz, CDCl$_3$): **δ** 161.85, 159.67, 153.58, 132.83, 130.68, 128.91, 121.94, 120.86, 96.48, 86.10.

2-Chloro-4-(2-furyl)pyrimidine (11)[12]

Compound prepared as described in literature[12]. While under N_2 pressure and a temperature of -10 °C, of *n*-butyllithium (2.5 M, 2.6 mL, 6.5 mmol) was added to excess furan (1.70 g, 25 mmol) dissolved in THF (10 mL). The reaction mixture was held at a steady temperature of -10 °C for 2 hours. Then, a solution of 2-chloropyrimidine **7** (0.69 g, 6.0 mmoles) in THF (3 mL) was added to the above mixture. The progress of the reaction was monitored by TLC on silica gel, eluting with dichloromethane:hexanes (1:1). Workup according to general method described above yielded 0.17 g (0.95 mmol, 25%) of 2-chloro-4-(2-furanyl)pyrimidine **(11)**.

This compound had mp 102–104 °C[12]; ^{1}H-NMR (400 MHz, CDCl$_3$): **δ** 8.35 (d, 1H, J = 4.8 Hz),

7.55 (m, 1H), 7.15 (t, 1H, J = 3.2 Hz), 6.86 (d, 1H, J = 4.8 Hz), 6.53 (m, 1H).

Compared to literature values of mp 102-104 °C; ^{1}H-NMR (Free base, 300 MHz, CDCl$_3$): **δ** 8.59

(d, 1H, J = 5.4 Hz), 7.63 (d, 1H, J = 1.8 Hz), 7.53 (d, 1H, J = 5.4 Hz), 7.39 (d, 1H, J = 3.6 Hz),

6.61 (dd, 1H, J = 3.6 Hz, 1.8 Hz).

2-Chloro-4-(biphen-2-yl)pyrimidine (12)

While under N$_2$ pressure and a temperature of -78 °C, (2.5 M, 0.8 mL, 2 mmol) of *n*-

butyllithium was added to 2-bromobiphenyl (0.47 g, 2 mmol) dissolved in THF (5 mL).Then, a

solution of 2-chloropyrimidine **7** (0.23 g, 2 mmol) dissolved in THF (3 mL) was added. The

progress of the reaction was monitored by TLC on silica gel, eluting with

dichloromethant:hexanes (1:1). The mixture was and allowed to reach -30 °C over 2 hours.

Workup according to general method described above yielded 0.12 g (44 mmol, 51%) of 2-

chloro-4-(biphenyl)pyrimidine **(12)**.

This compound was a yellow oil; ^{1}H-NMR (400 MHz, CDCl$_3$): **δ** 8.23 (d, 1H, J = 5.2 Hz), 7.83

(t, 1H, J = 7.6 Hz), 7.53 (m, 2H), 7.46 (d, 1H, J = 1.6 Hz), 7.32 (m, 3H), 7.19 (m, 2H), 6.70 (d,

1H, J = 5.2 Hz).

2-Chloro-4,6-bis(2-phenylethynyl)pyrimidine (14)

While under N$_2$ pressure and a temperature of -78 °C, (2.5 M, 1.57 mL, 3.9 mmoles) of

n-butyllithium was added to phenylacetylene (0.40 g, 3.9 mmol) dissolved in THF (10 mL). The

mixture was held at a steady temperature of -78 °C for 30 minutes. Then, a solution of 2-

chloropyrimdine **7** (0.30 g, 2.62 mmol) in THF (3 mL) was added to the mixture. The

temperature was allowed to reach -30 °C over 3 hours. Workup according to general method described above yielded 0.15 g (0.47 mmol, 37%) of 2-chloro-4,6-bis(2-phenylethynyl)pyrimidine **(14)**.

This compound had a mp 150-152 °C, yield 37 %; ^{1}H-NMR (300 MHz, CDCl$_3$): **δ** 7.64 (m, 4H) 7.52 (s, 1H), 7.38 (m, 6H). ^{13}C-NMR (300 MHz, CDCl$_3$): **δ** 161.78, 153.42, 132.87, 130.75, 128.94, 124.07, 120.86, 96.98, 86.09.

2-(4-Methylpiperazino)-4-(2-phenylethynyl)pyrimidine dihydrobromide (16•2HBr•H$_2$O)

To the 2-chloropyrimidine product **10** (0.05 g, 0.23 mmol), dissolved in toluene (1.5 mL), excess 1-methylpiperazine (0.07 g, 6.7 mmol) was added. The reaction was allowed to react at room temperature for 24 hours. Workup corresponding to general method described above yielded 0.0014 g (0.005 mmol, 2.1 %) of **16**.

This compound had mp 193-195 °C; ^{1}H-NMR (Free base, 300 MHz, CDCl$_3$): **δ** 8.29 (d, 1H, $J =$ 4.8 Hz), 7.59 (d, 2H, $J =$ 4 Hz), 7.36 (m, 3H), 6.67 (d, 1H, $J =$ 4.8 Hz), 3.88 (t, 4H, $J =$ 7.2 Hz), 2.47 (t, 4H, $J =$ 4.8 Hz), 2.34 (s, 3H). ^{13}C-NMR (Free base, 300 MHz, CDCl$_3$): **δ** 161.86, 158.10, 151.41, 132.53, 129.70, 128.67, 121.95, 112.61, 91.58, 87.97, 55.23, 46.51, 43.93.

Analysis. Calculated for C$_{17}$H$_{18}$N$_4$•2HBr•H$_2$O: C, 44.56, H, 4.84, N, 12.23. Found: C, 44.08, H, 4.82, N, 11.94.

2-Morpholino-4-(2-phenylethynyl)pyrimidine dihydrobromide (17•2HBr•0.5H$_2$O)

To the 2-chloropyrimidine product **10** (0.058 g, 0.27 mmol) dissolved in toluene (1.5 mL), excess morpholine (0.70 g, 8.07 mmol) was added and refluxed. After 15 minutes, workup corresponding to general method yielded 0.074 g (0.28 mmol, 11 %) of **17**.

This compound had mp 207-209 °C; ^{1}H-NMR (Free base, 400 MHz, CDCl$_3$): **δ** 8.33 (d, 1H, $J =$ 5.1 Hz), 7.62 (m, 2H), 7.41 (t, 3H, $J =$ 3.0 Hz), 6.74 (d, 1H, 5.1), 3.85 (m, 4H), 3.80 (m, 4H). ^{13}C-NMR (Free base, 300 MHz, CDCl$_3$): **δ** 161.86, 158.10, 151.42, 132.54, 129.71, 128.68, 121.96, 112.61, 91.58, 87.97, 55.28, 43.92.

Analysis. Calculated for C$_{16}$H$_{21}$N$_3$O•2HBr•0.5H$_2$O: C, 44.06; H, 4.16; N, 9.63. Found: C, 43.09; H, 3.97; N, 9.53.

4-(2-Phenylethynyl)-2-(piperazino)pyrimidine (18)

To the 2-chloropyrimidine product **10** (0.150 g, 0.699 mmol), dissolved in toluene (1.5 mL), excess piperazine (0.60 g, 8.07 mmol) was added and refluxed. After 15 minutes, workup corresponding to general method described above yielded 0.02 g (0.005 mmol, 3.34 %) of **18**.

This compound was a yellow oil; ^{1}H-NMR (400 MHz, CDCl$_3$): **δ** 8.31 (d, 1H, $J =$ 4.8 Hz), 7.59 (m, 2H), 7.38 (t, 3H, $J =$ 1.2 Hz), 6.66 (d, 1H, $J =$ 4.8 Hz), 3.84 (m, 4H), 2.94 (m, 4H).

4-(2-Furyl)-2-(piperazino)pyrimidine (19)

At room temperature, excess piperazine (0.05 g, 0.63 mmol) was added to 2-chloropyrimidine product **11** (0.32 g, 1.75 mmol), dissolved in toluene (1 mL) and of methanol (1 mL). The mixture was refluxed for 2 hours and allowed to react at room temperature for 24 hours. Workup corresponding to general method described above yielded 0.05 g (0.22 mmol, 95 %) of **19**.

This compound was a brown oil; ^{1}H-NMR (Free base, 400 MHz, CDCl$_3$): **δ** 8.39 (d, 1H, $J =$ 5.2 Hz), 7.57 (m, 1H), 7.20 (m, 1H), 6.91 (d, 1H, $J =$ 5.2 Hz), 6.56 (s, 1H), 3.99 (s, 4H), 2.05 (s, 1H).

4-(2-Furyl)-2-(4-methylpiperazino)pyrimidine dihydrobromide (20•1.75HBr•0.25H$_2$O)[13]

Compound prepared as described in literature[13]. At room temperature, excess 1-methylpiperazine (0.18 g, 1.83 mmol) was added to the pyrimidine product **11** (0.11 g, 0.61 mmol) dissolved in toluene (1mL). After 24 hours, workup corresponding to general method described above yielded 0.14 g (0.58 mmol, 78 %) of **20**.

This compound had a mp 234-236 °C; ^{1}H-NMR (Free base, 400 MHz, CDCl$_3$): **δ** 8.34 (d, 1H, J = 5.2 Hz), 7.54 (m, 1H), 7.15 (t, 1H, J = 3.6 Hz), 6.86 (d, 1H, J = 5.2 Hz), 6.54 (m, 1H), 3.90 (t, 4H, J = 5.2 Hz), 2.48 (t, 4H, J = 5.2 Hz), 2.34 (s, 3H). ^{13}C-NMR (Free base, 400 MHz, CDCl$_3$): **δ** 161.64, 158.29, 155.85, 152.69, 144.35, 112.08, 111.17, 103.93, 54.99, 46.26, 43.64.

Compared to literature values of mp 270-273 °C; ^{1}H-NMR (Free base, 300 MHz, CDCl$_3$): **δ** 8.38 (d, 1H, J = 5.5 Hz), 7.60 (m, 1H), 6.88 (d, 1H, J = 5.5 Hz), 6.55 (m, 1H), 3.95 (m, 4h), 2.50 (m, 4H), 2.40 (s, 3H).

Analysis. Calculated for C$_{13}$H$_{16}$N$_4$O•1.75HBr•0.25H$_2$O: C, 40.00; H, 4.71; N, 14.35; Found: C, 40.43; H, 4.70; N, 14.31.

4-(Biphen-2-yl)-2-(4-methylpiperazino)pyrimidine dihydrobromide (21•2HBr•H$_2$O)

At room temperature, excess 1-methylpiperazine (0.13 g, 1.31 mmol) was added to the pyrimidine product **12** (0.12 g, 0.44 mmol) dissolved in toluene (1 mL). After 24 hours, workup corresponding to general method described above yielded 0.12 g (0.35 mmol, 86 %) of **21**.

This compound had a mp 85-87 °C; ^{1}H-NMR (Free base, 400 MHz, CDCl$_3$): **δ** 8.09 (d, 1H, J = 5.0 Hz), 7.70 (s, 1H), 7.44 (m, 3H), 7.25 (m, 3H), 6.29 (d, 1H, J = 5.0 Hz), 3.68 (d, 4H, J = 4.0 Hz), 2.37 (t, 4H, J = 4.8 Hz), 2.04 (s, 1H). ^{13}C-NMR (Free base, 400 MHz, CDCl$_3$): **δ** 166.87,

161.56, 157.00, 141.65, 141.33, 137.89, 130.95, 130.10, 129.39, 128.06, 127.54, 126.76, 110.48, 54.98, 46.28, 43.53, 14.22.

Analysis. Calculated for $C_{21}H_{22}N_4 \cdot 2HBr \cdot H_2O$: C, 49.43; H, 5.14; N, 10.98. Found: C, 48.32; H, 5.0; N, 10.57.

4-(2-Furyl)-*N*-(2-(pyrrolidino)ethyl)pyrimidine-2-amine dihydrobromide (23•2HBr)

At room temperature, excess 1-(2-aminoethyl)-pyrrolidine (0.47 g, 4.1 mmol) was added to pyrimidine product **11** (0.12 g, 0.69 mmol) dissolved in toluene (1 mL). The mixture was refluxed for 16 hours. Workup corresponding to general method described above yielded 0.13 g (0.49 mmol, 27 %) of **23**.

This compound had a mp 173-175 °C; ^{1}H-NMR (Free base, 400 MHz, CDCl$_3$): **δ** 8.30 (d, 1H, *J* = 5.2 Hz), 7.55 (t, 1H, *J* = 1.6 Hz), 7.14 (d, 1H, *J* = 3.6 Hz.), 6.87 (m, 1H), 6.53 (m, 1H), 5.72 (s, 1H), 3.57 (m, 2H), 2.72 (t, 2H, *J* = 6.4 Hz), 2.56 (m, 4H), 1.78 (m, 4H).

^{13}C-NMR (Free base, 400 MHz, CDCl$_3$): **δ** 162.39, 158.50, 156.11, 152.39, 144.43, 112.10, 111.33, 104.50, 54.82, 53.90, 40.17, 23.54.

Analysis. Calculated for $C_{14}H_{18}N_4O \cdot 2HBr$: C, 40.02; H, 4.80; N, 13.34. Found: C, 40.15; H, 4.90; N, 13.30.

2-(4-Methylpiperazino)-4,6-bis(2-phenylethynyl)pyrimidine dihydrobromide (25•2HBr•0.5H₂O)

At room temperature, excess 1-methylpiperazine (0.05 g, 0.48 mmol) was added to 2-chloropyrimidine product **14** (0.05 g, 0.16 mmol). After 24 hours, the reaction progress was

monitored by TLC, eluting with dichloromethane:hexanes (1:1). Workup corresponding to general method described above yielded 0.0014 g (0.004 mmol, 2.9 %) of **25**.

This compound had mp 167-169°C; ^{1}H-NMR (Free base, 300 MHz, CDCl$_3$): **δ** 7.63 (m, 4H), 7.43 (m, 6H), 6.92 (s, 1H), 3.96 (t, 4H, J = 9.9), 2.51 (t, 4H, J = 9.9), 2.38 (s, 3H).

^{13}C-NMR (Free base, 400 MHz, CDCl$_3$): **δ** 161.96, 151.65, 132.58, 129.80, 128.71, 121.91, 115.27, 92.14, 87.80, 55.27, 46.48, 43.98.

Analysis. Calculated for C$_{25}$H$_{22}$N$_4$•2HBr•0.5H$_2$O: C, 54.66; H, 4.59; N, 10.20. Found: C, 54.85; H, 4.39; N, 10.21.

1,4-Bis[4-(furan-2-yl)pyrimidin-2-yl]piperazine dihydrobromide (28•2HBr•H$_2$O)

To the pyrimidine product **11** (0.17 g, 0.95 mmol), dissolved in toluene (1.5 mL), excess piperazine (0.02 g, 0.20 mmol) was added and refluxed for 2 hours. The reaction progress was monitored by TLC, eluting with dichloromethane. Workup corresponding to general method described above yielded 0.004 g (0.007 mmol, 23 %) of **28**.

This compound had a mp 328-330 °C; ^{1}H-NMR (Free base, 400 MHz, CDCl$_3$): **δ** 8.38 (d, 1H, J =5.2 Hz), 7.57 (t, 2H, J = 1.6 Hz), 7.19 (d, 2H, J = 3.2 Hz), 6.89 (d, 2H, J = 4.8 Hz), 6.55 (m, 2H), 3.99 (s, 8H). ^{13}C-NMR (Free base, 400 MHz, CDCl$_3$): **δ** 161.76, 158.38, 155.94, 152.68, 144.44, 112.15, 111.30, 104.15, 43.69.

Analysis. Calculated for C$_{20}$H$_{18}$N$_6$O$_3$•2HBr•H$_2$O: C, 44.06; H, 3.88; N, 15.41. Found: C, 44.10; H, 3.26; N, 15.22.
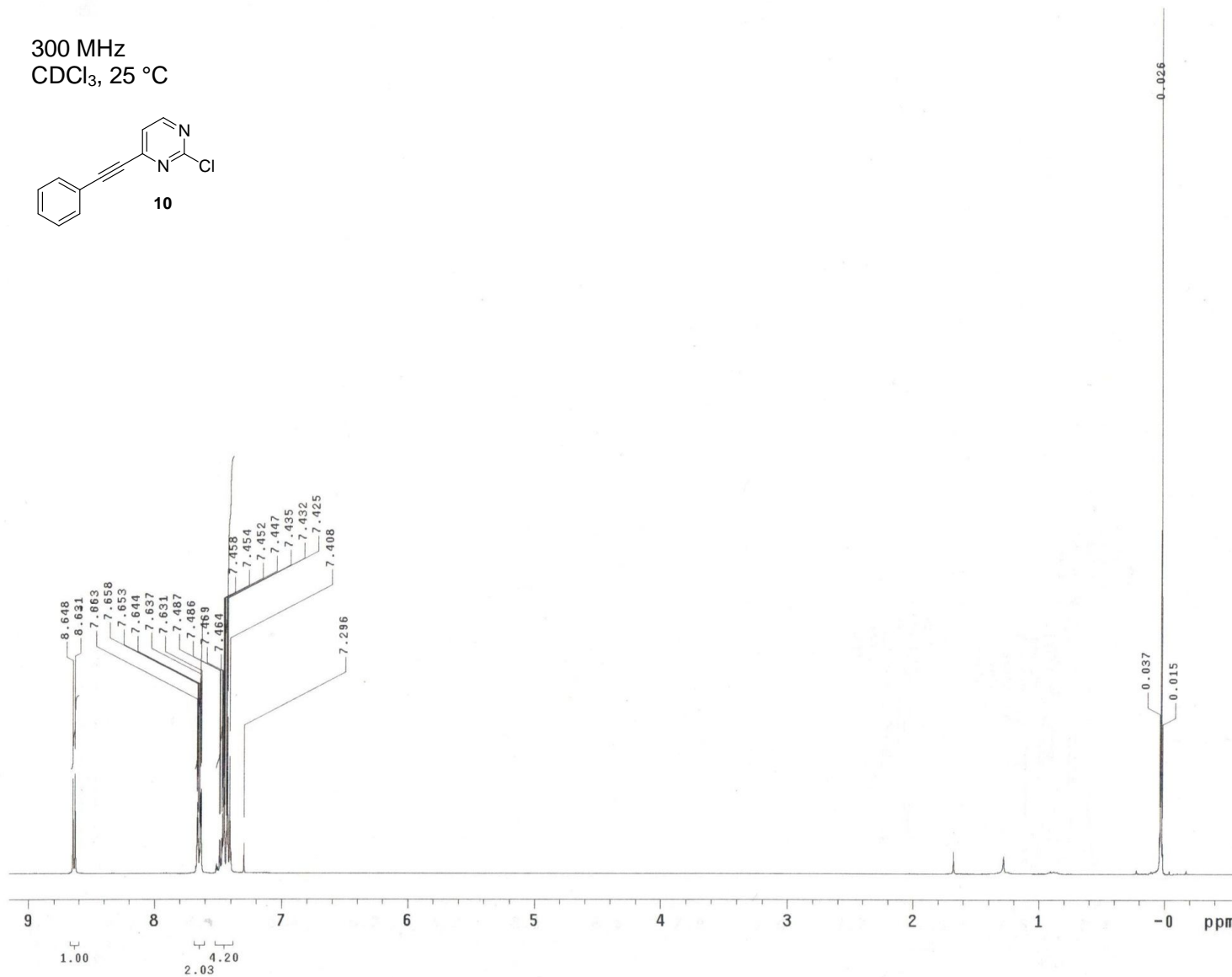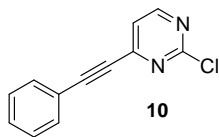
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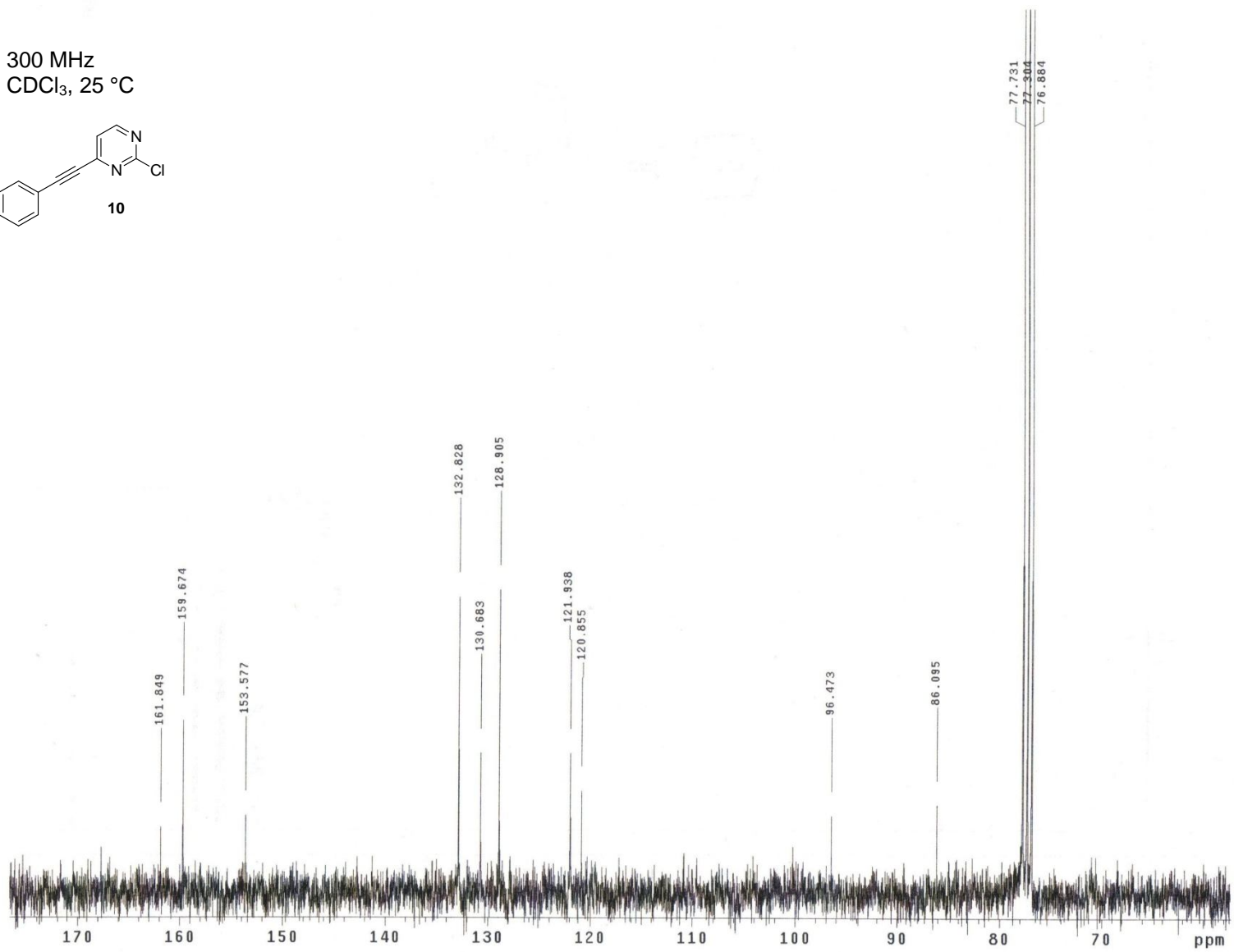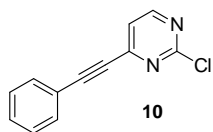
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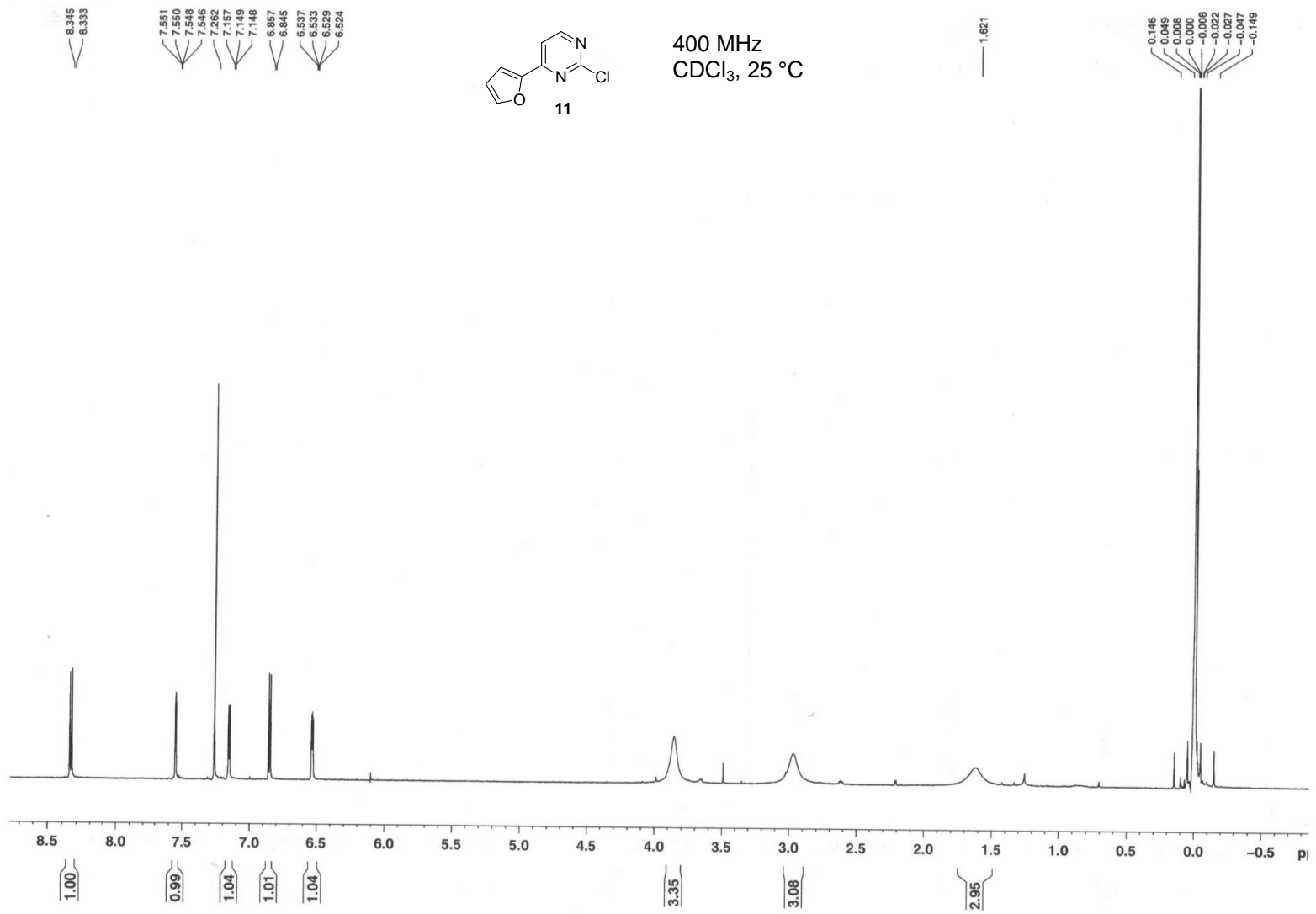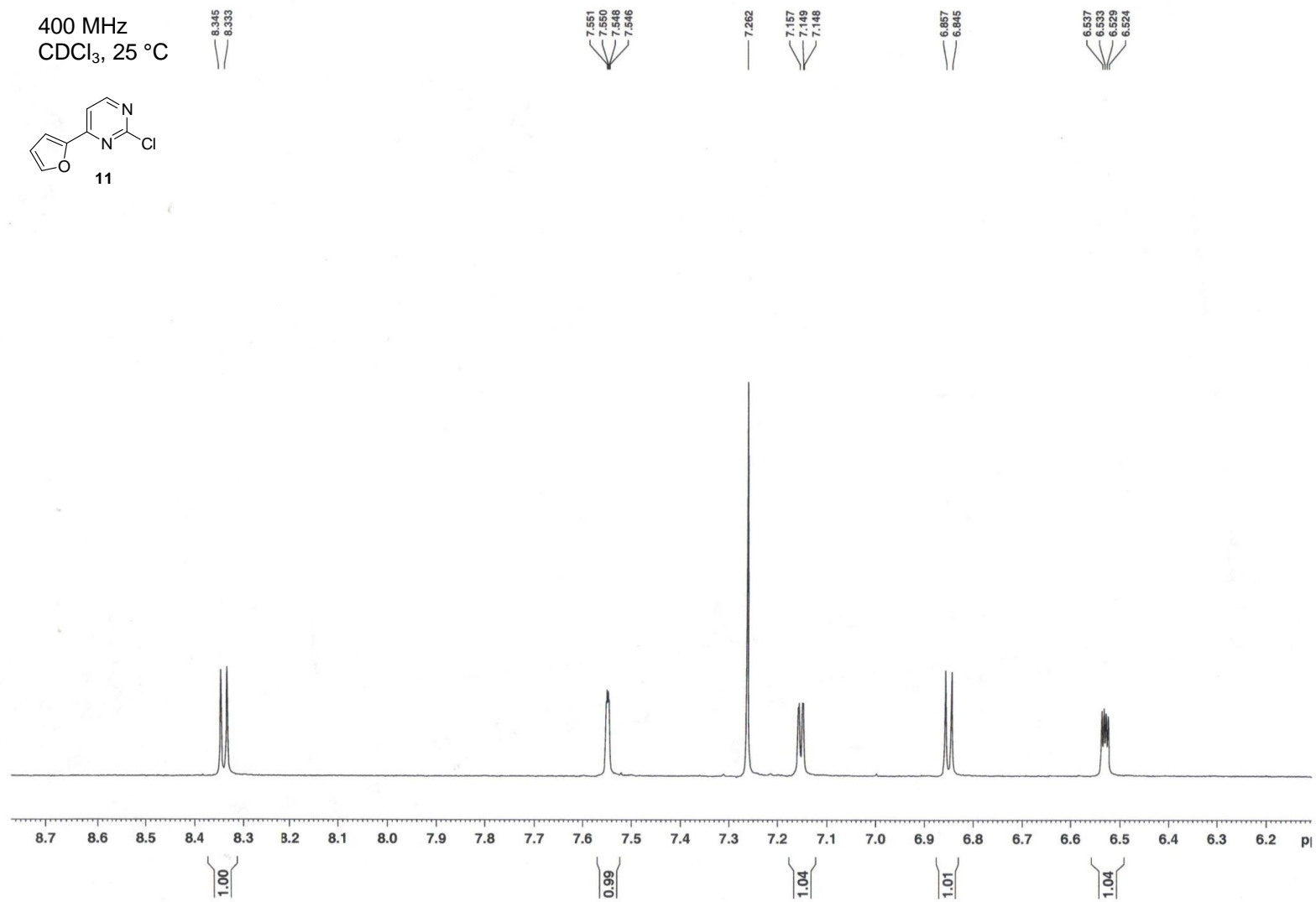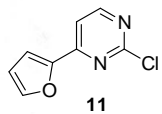
APPENDIX

300 MHz
CDCl$_3$, 25 °C

10

8.648
8.621
7.663
7.658
7.653
7.644
7.637
7.631
7.487
7.486
7.469
7.464
7.458
7.454
7.452
7.447
7.435
7.432
7.425
7.408
7.296
0.037
0.026
0.015

9   8   7   6   5   4   3   2   1   -0   ppm

1.00   2.03   4.20

300 MHz
CDCl$_3$, 25 °C

77.731
77.304
76.884

132.828
128.905

159.674

130.683

121.938
120.855

161.849

153.577

96.473

86.095

170   160   150   140   130   120   110   100   90   80   70   ppm

A2

8.345
8.333

7.551
7.550
7.548
7.546
7.262
7.157
7.149
7.148
6.857
6.845
6.537
6.533
6.529
6.524

1.621

0.146
0.049
0.008
0.000
-0.008
-0.022
-0.027
-0.047
-0.149

400 MHz
CDCl₃, 25 °C

11

1.00

0.99

1.04

1.01

1.04

3.35

3.08

2.95

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   −0.5   p

A3

400 MHz
CDCl$_3$, 25 °C

8.345
8.333

7.551
7.550
7.548
7.546

7.262

7.157
7.149
7.148

6.857
6.845

6.537
6.533
6.529
6.524

11

8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 6.4 6.3 6.2 ppm
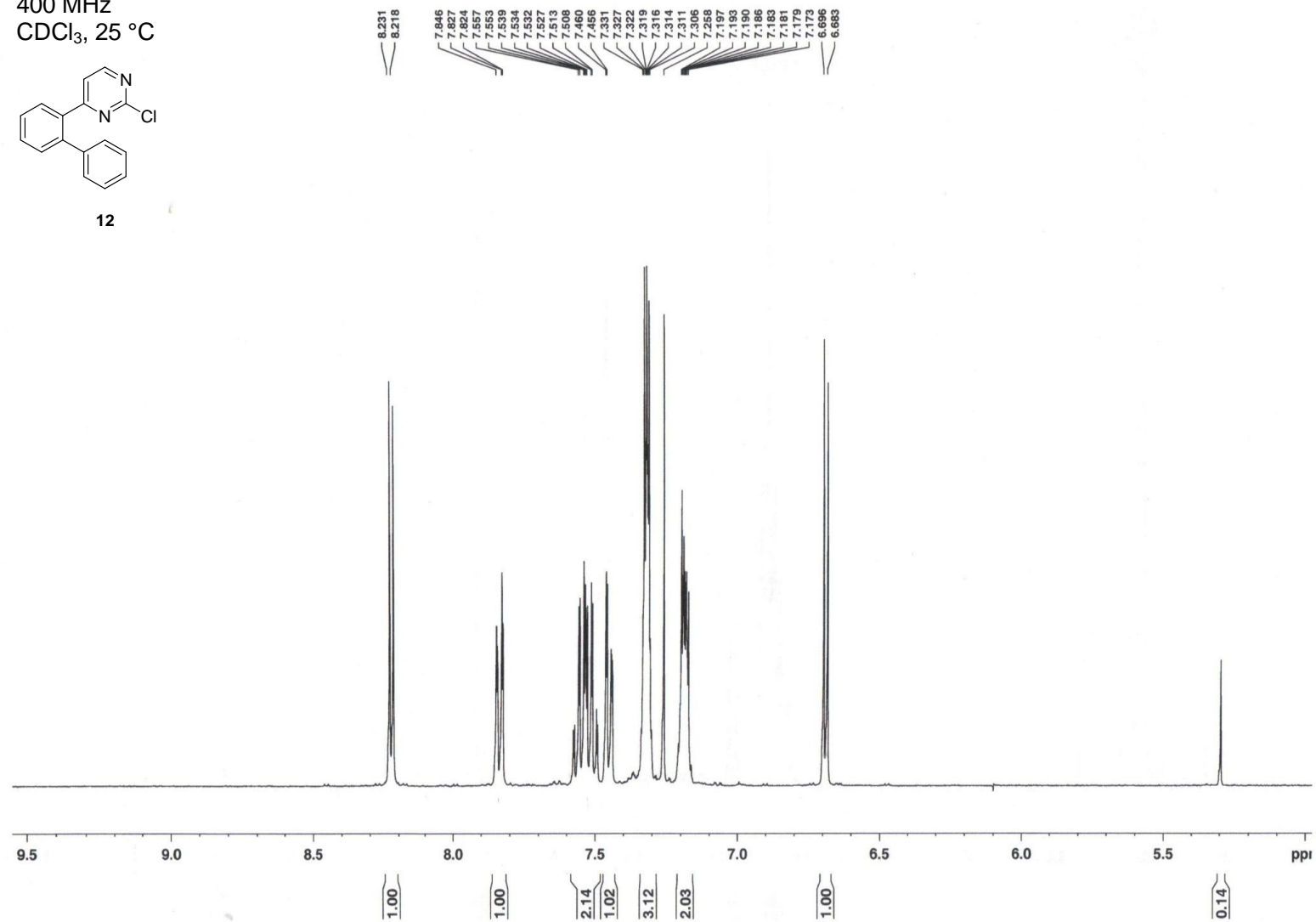
1.00   0.99   1.04   1.01   1.04

400 MHz
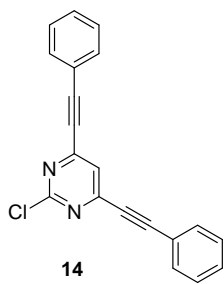CDCl₃, 25 °C

12

A5

400 MHz
CDCl₃, 25 °C

12

A6

300 MHz
CDCl₃, 25 °C

STANDARD 1H OBSERVE

SAMPLE
date   Aug   6 2006
solvent   CDCl3
file   exp

ACQUISITION
sfrq   299.949
tn   H1
at   4.096
np   32768
sw   4000.0
fb   2200
bs   2
ss   2
tpwr   58
pw   6.7
d1   1.000
tof   0
nt   128
ct   34
alock   n
gain   not used

FLAGS
il   n
in   n
dp   y
hs   nn

DISPLAY
sp   -87.8
wp   2580.2
vs   532
sc   0
wc   250
hzmm   10.32
is   500.00
rfl   500.1
rfp   0
th   20
ins   1.000
sen   0
lw   0
nm   ph

DEC. & VT
dfrq   299.949
dn   H1
dpwr   30
dof   0
dm   nnn
dmm   c
dmf   200
dseq
dres   1.0
homo   n

PROCESSING
lb   0.10
wtfile
proc   ft
fn   65536
math   f
werr
wexp
wbs
wnt

14

3.90 6.14
1.00

0.51

A7

300 MHz
CDCl₃, 25 °C

14

7.669
7.667
7.644
7.640
7.559
7.556
7.560
7.474
7.465
7.462
7.478
7.439
7.498
7.497
7.419
7.417
7.414
7.296

7.80 7.75 7.70 7.65 7.60 7.55 7.50 7.45 7.40 7.35 7.30 ppm

3.90

1.00

6.14

300 MHz
CDCl$_3$, 25 °C

14

132.873
130.752
128.936

161.780
153.424
124.067
120.855
96.977
86.087
77.724
77.304
76.877
0.268

220   200   180   160   140   120   100   80   60   40   20   0   ppm

A9

300 MHz
CDCl₃, 25 °C

16

A10

300 MHz
CDCl$_3$, 25 °C

16

161.856
158.102
151.409
132.530
129.699
128.669
121.953
112.605
91.574
87.972
77.724
77.304
76.877
55.227
46.513
43.926
0.261

220 200 180 160 140 120 100 80 60 40 20 0 ppm

A11

400 MHz
CDCl$_3$, 25 °C

8.351
8.334
7.640
7.634
7.617
7.616
7.60?
7.416
7.413
7.406
7.392
6.742
6.725
3.885
3.872
3.866
3.854
3.812
3.801
3.795
3.782
0.031
0.031

8   7   6   5   4   3   2   1   ppm

0.97   1.96   2.98   1.00   4.18   4.07

A12

400 MHz
CDCl₃, 25 °C

18

8.307
8.291
7.610
7.604
7.585
7.578
7.384
7.380
7.372
7.366
7.364
7.361
7.360
7.221
6.672
6.656
4.131
4.108
3.857
3.841
3.824
2.960
2.944
2.927
2.043
1.281
1.257
1.233
0.011
-0.011
0.000

11   10   9   8   7   6   5   4   3   2   1   -0   -1 ppm

1.00   3.07   1.00   0.88   2.93   1.38   1.46
     1.98   4.03   1.33

A13

400 MHz
CDCl$_3$, 25 °C

8.389
8.376
7.574
7.572
7.570
7.568
7.206
7.205
7.198
7.196
6.907
6.894
6.564
6.560
6.556
6.551
4.156
4.138
4.120
4.103
3.995
3.496
2.177
2.052
1.284
1.266
1.248
0.154
0.080
0.056
0.022
0.016
0.008
-0.000
-0.040
-0.141

1.00
0.92
0.36
0.91
0.96
0.95
0.41
3.91
0.39
0.12
0.61
1.28

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 −0.5 ppm

A14

400 MHz
CDCl$_3$, 25 °C

20

8.338
8.325

7.542
7.539
7.287
7.156
7.148
7.147
6.856
6.843
6.527
6.523
6.519
6.515

3.910
3.897
3.885

2.495
2.482
2.470
2.340
2.159

-0.000
-0.008

| 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 | −0.5 | pp |

1.00

0.98

0.98

0.98

0.98

1.10

4.50
3.10
0.18

A15

400 MHz
CDCl₃, 25 °C

161.64
158.29
155.85
152.69
144.35
112.08
111.17
103.93
77.39
77.08
76.76
54.99
46.26
43.64
−0.00

20

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10 ppm
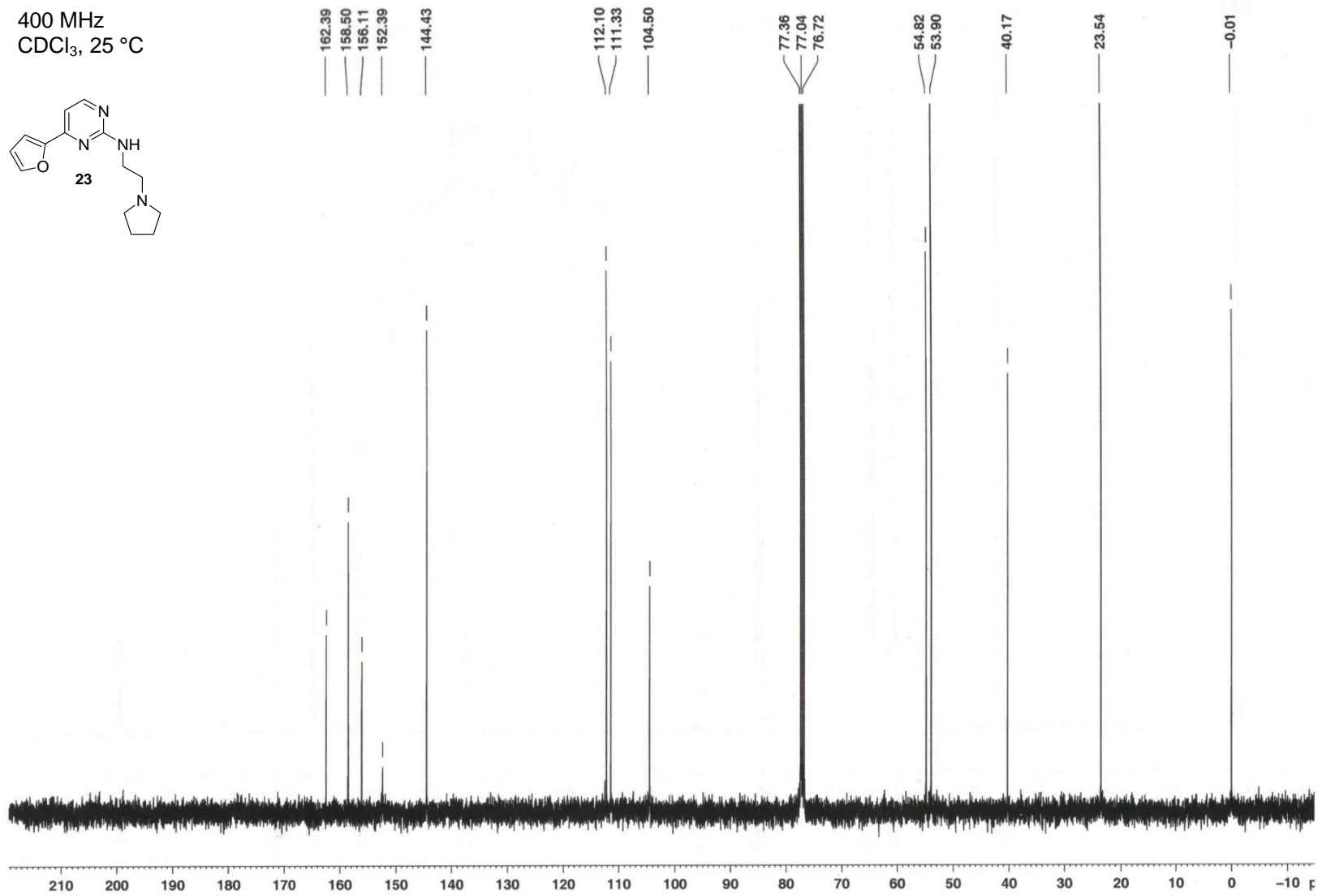
A16

400 MHz
CDCl₃, 25 °C

21

A17

400 MHz
CDCl₃, 25 °C

166.87
161.56
157.00
141.65
141.33
137.89
130.95
130.10
129.39
128.06
127.54
126.76
110.48
77.37
77.05
76.73
54.98
46.28
43.53
14.22
0.01

21

400 MHz
CDCl$_3$, 25 °C

8.302
8.289

7.551
7.549
7.547
7.272
7.138
7.129
6.867
6.865
6.854
6.852
6.533
6.530
6.529
6.526
6.522
6.520
6.518

3.581
3.567
3.552
3.537

2.735
2.719
2.704
2.577
2.562
2.550
2.547

1.796
1.789
1.781
1.772
1.765

−0.001
−0.002
−0.009

23

1.00
0.96
0.95
0.98
0.98
0.97
0.03
2.02
2.21
4.35
4.21

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 p

A19

400 MHz
CDCl₃, 25 °C

162.39
158.50
156.11
152.39
144.43
112.10
111.33
104.50
77.36
77.04
76.72
54.82
53.90
40.17
23.54
−0.01

23

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10 p

A20

300 MHz
CDCl₃, 25 °C

25

7.647
7.641
7.633
7.624
7.615
7.425
7.403
6.923
3.971
3.955
3.930
2.538
2.521
2.505
2.382
1.290
0.035

7   6   5   4   3   2   1   ppm

4.00   0.92   4.0l   4.10
  6.10   3.22

400 MHz
CDCl₃, 25 °C

25

161.955
151.654
132.576
129.798
128.707
121.908
115.269
92.139
87.804
77.716
77.289
76.869
55.265
46.482
43.979
0.253

400 MHz
CDCl₃, 25 °C

8.382
8.369
7.568
7.566
7.564
7.261
7.199
7.191
6.900
6.888
6.558
6.553
6.549
6.545
3.987
1.584
0.012
0.000
−0.008

28

2.00
1.97
2.02
2.03
2.03
8.29

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 ppm

400 MHz
CDCl₃, 25 °C

28