C-O BOND FORMATION ON C-5 OF 2,4-DIAMINOPYRIMIDINES

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การสร้างพันธะระหว่างคาร์บอนและออกซิเจนบนคาร์บอนตำแหน่งที่ 5 ของ 2,4-ไดอะมิโนไพริมิดีน

นายณัฐกร อุปถัมภ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ณัฐกร อุปถัมภ์ : การสร้างพันธะระหว่างคาร์บอนและออกซิเจนบนคาร์บอนตำแหน่งที่ 5 ของ 2,4-ไดอะมิโนไพริมิดีน. (C-O BOND FORMATION ON C-5 OF 2,4-DIAMINOPYRIMIDINES) อ.ที่ปรึกษาหลัก : ผศ. ดร.วรวรรณ พันธุมนาวิน, อ.ที่ปรึกษาร่วม : ศ. ดร.ธีรยุทธ วิไลวัลย์

งานวิจัยนี้มีจุดประสงค์เพื่อหาสภาวะที่มีประสิทธิภาพสำหรับปฏิกิริยาออกซิเดชันของสารประกอบ 2,4-ไดอะมิโนไพริมิดีนให้เป็นอนุพันธ์ที่มีหมู่ไฮดร็อกซีบนคาร์บอนตำแหน่งที่ 5 ถึงแม้ว่าจะมีรายงานการทำปฏิกิริยาบอยแลนด์-ซิมส์ ออกซิเดชันของสารประกอบกลุ่มนี้ แต่วิธีการนั้นจะให้ปริมาณผลได้ของสารผลิตภัณฑ์ที่ต่ำและทำซ้ำให้ได้ผลเช่นเดิมได้ยาก ดังนั้นในงานวิจัยนี้จึงได้ศึกษาหาสภาวะที่เหมาะสมที่สุดอีกครั้งในการทำปฏิกิริยาบอยแลนด์-ซิมส์ออกซิเดชันของสารประกอบ 2,4-ไดอะมิโนไพริมิดีน รวมถึงรายงานผลของตัวแปรต่าง ๆ ต่อปริมาณผลได้ของสารผลิตภัณฑ์ที่มีหมู่ไฮดร็อกซีบนคาร์บอนตำแหน่งที่ 5 จากปฏิกิริยาออกซิเดชัน ซึ่งได้ศึกษาการเกิดปฏิกิริยาโดยใช้ 2,4-ไดอะมิโน-6-เอทิลไพริมิดีนเป็นสารต้นแบบ ในมาตราส่วน 0.10 มิลลิโมล และคำนวณปริมาณผลได้ของสารผลิตภัณฑ์โดยใช้เทคนิคโปรตอนเอ็นเอ็มอาร์สเปกโทรสโกปีเปรียบเทียบกับสารมาตรฐาน จากการทดลองทำปฏิกิริยาออกซิเดชันในมาตราส่วนที่ใหญ่ขึ้น ของ 2,4-ไดอะมิโนไพริมิดีนที่ไม่มีหมู่แทนที่ใด ๆ บนคาร์บอนตำแหน่งที่ 6 และอนุพันธ์ของสารประกอบ 2,4-ไดอะมิโนไพริมิดีนที่มีหมู่แอลคิลซึ่งเป็นหมู่ให้อิเล็กตรอน เช่น หมู่เอทิล และหมู่ไอโซโพรพิลบนคาร์บอนตำแหน่งที่ 6 ด้วยสภาวะที่เหมาะสม พบว่า ปริมาณผลได้ของสารผลิตภัณฑ์ที่มีหมู่ไฮดร็อกซีบนคาร์บอนตำแหน่งที่ 5 อยู่ในระดับที่น่าพึงพอใจ (43-58 เปอร์เซ็นต์) อย่างไรก็ตาม อนุพันธ์ของสารประกอบ 2,4-ไดอะมิโนไพริมิดีนที่มีหมู่ดึงอิเล็กตรอน เช่น หมู่คลอโร และหมู่ไตรฟลูออโรเมทิลบนคาร์บอนตำแหน่งที่ 6 ไม่เกิดปฏิกิริยาภายใต้สภาวะเดียวกัน ในงานวิจัยจึงได้ศึกษาปฏิกิริยาออกซิเดชันของ 2,4-ไดอะมิโนไพริมิดีนที่มีหมู่ดึงอิเล็กตรอนเพิ่มเติมด้วยตัวออกซิไดส์อื่น ๆ โดยใช้อนุพันธ์ของ 2,4-ไดอะมิโนไพริมิดีนที่มีหมู่ไตรฟลูออโรเมทิลบนคาร์บอนตำแหน่งที่ 6 เป็นสารต้นแบบ เนื่องจากหมู่ไตรฟลูออโรเมทิลทำให้ติดตามความก้าวหน้าของปฏิกิริยาได้อย่างสะดวกด้วยเทคนิคฟลูออรีนเอ็นเอ็มอาร์สเปกโทรสโกปี จากการทดลองทำปฏิกิริยาของ 2,4-ไดอะมิโน-6-ไตรฟลูออโรเมทิลไพริมิดีนกับตัวออกซิไดส์และสภาวะในการทำปฏิกิริยาที่หลากหลายในมาตราส่วน 0.05 มิลลิโมล พบว่า ไม่ได้สารผลิตภัณฑ์ที่มีหมู่ไฮดร็อกซีบนคาร์บอนที่ 5 ตามที่คาดหวังไว้ อย่างไรก็ตาม สารผลิตภัณฑ์ข้างเคียง เช่น สารประกอบ*เอ็น*-ออกไซด์ และ 2,4-ไดอะมิโนไพริมิดีนที่มีหมู่ฮาโลเจนบนคาร์บอนตำแหน่งที่ 5 ได้ถูกแยกออกมาและพิสูจน์เอกลักษณ์อย่างเต็มรูปแบบ ดังนั้นในงานวิจัยนี้ได้รายงานสภาวะที่เหมาะสมที่สุดในการทำปฏิกิริยาบอยแลนด์-ซิมส์ ออกซิเดชันของสารประกอบ 2,4-ไดอะมิโนไพริมิดีน และได้แสดงให้เห็นว่าปฏิกิริยานี้สามารถใช้ได้กับ 2,4-ไดอะมิโนไพริมิดีนที่มีความหนาแน่นของอิเล็กตรอนสูงเท่านั้น ในขณะนี้การทำปฏิกิริยาออกซิเดชันของ 2,4-ไดอะมิโนไพริมิดีนที่มีความหนาแน่นของอิเล็กตรอนต่ำยังคงความท้าทายและยังไม่สามารถทำได้สำเร็จด้วยวิธีใดก็ตาม

สาขาวิชา	เคมี	ลายมือชื่อนิสิต .....
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Natthakorn Uppatam : C-O BOND FORMATION ON C-5 OF 2,4-DIAMINOPYRIMIDINES. Advisor: Asst. Prof. Worawan Bhanthumnavin, Ph.D. Co-advisor: Prof. Tirayut Vilaivan, Ph.D.

This work aims to find a robust oxidation condition that can efficiently convert 2,4-diaminopyrimidines to its C5-hydroxylated derivative. Although Boyland-Sims oxidation of these substrates has been reported, this procedure suffers low yield and poor reproducibility. The conditions for Boyland-Sims oxidation of 2,4-diaminopyrimidines were thus re-optimized. The parameters that could affect the yields of the 5-hydroxy products were investigated using 2,4-diamino-6-ethylpyrimidine as a model compound. The reactions were performed at 0.10 mmol scale of the starting pyrimidine. The yields of the products were monitored by ^{1}H NMR spectroscopy with reference to an internal standard. The reaction was reproduced at larger scale employing the optimized condition. The 5-hydroxylated products were obtained in satisfactory yields (43-58%) from the oxidation of 2,4-diaminopyrimidines without substituent or with an electron-donating alkyl group such as ethyl and isopropyl group at the C-6 position. However, the same conditions gave no oxidation product with electron-deficient 2,4-diaminopyrimidines bearing chloro or trifluoromethyl group at the C-6 position. Oxidation of the electron-deficient 2,4-diaminopyrimidines was further studied using other oxidants. The electron-deficient 6-trifluoromethyl substituted analogue was selected as a model compound, since the trifluoromethyl group facilitates reaction monitoring using ^{19}F NMR technique. The reactions were performed at 0.05 mmol scale of the fluorinated pyrimidine substrate with various oxidants. The expected C5-hydroxylated product was not obtained from the oxidation of 2,4-diamino-6-trifluoromethylpyrimidine under various conditions. However, side products such as the *N*-oxide and the 5-halogenated products were isolated and fully characterized. Therefore, this work has provided the optimized conditions for Boyland-Sims oxidation of 2,4-diaminopyrimidines and demonstrated that this oxidation is only suitable for electron rich 2,4-diaminopyrimidines. At present, oxidation of electron-deficient substrates still remains challenging, and could not be achieved by any methods.

Field of Study: Chemistry Student's Signature .....

Academic Year: 2018 Advisor's Signature .....

Co-advisor's Signature .....

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LIST OF ABBREVIATIONS

AIBN	azobisisobutyronitrile
br	broad signal (NMR)
brs	broad singlet (NMR)
CCA	α-cyano-4-hydroxycinnamic acid
$CDCl_3$	deuterated chloroform
CNS	central nervous system
d	doublet (NMR)
dd	doublet of doublet (NMR)
DCE	dichloroethane
DCM	dichloromethane
DHFR	dihydrofolate reductase
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dTMP	deoxythymidine monophosphate
dUMP	deoxyuridine monophosphate
equiv.	equivalent
ESI-QTOF	Electrospray Ionization Quadrupole Time-of-Flight
g	gram
HFIP	hexafluoroisopropanol
HRMS	high resolution mass spectrometry
Hz	hertz
HOAc	acetic acid
*i*PrOH	isopropanol
J	coupling constant
K_a	acid dissociation constant
lm	lumen
m	multiplet (NMR)

M	molar
MALDI-TOF	Matrix Assisted Laser Desorption/Ionization Time-of-Flight
MeCN	acetonitrile
MeOH	methanol
mg	milligram
MHz	megahertz
mL	milliliter
mm	millimeter
mmol	millimole
mol	mole
MS	mass spectrometry
m/z	mass-to-charge ratio
NMR	Nuclear Magnetic Resonance
OAc	acetate
p-	*para*
PIDA	diacetoxyiodobenzene
PIFA	[bis(trifluoroacetoxy)iodo]benzene
PPA	propionic acid
ppm	part per million
PYR	pyrimethamine
q	quartet (NMR)
R_f	retention factor
s	singlet (NMR)
t	triplet (NMR)
Temp.	temperature
TBA-OH	tetrabutylammonium hydroxide
TFE	trifluoroethanol
THF	tetrahydrofuran
TLC	thin layer chromatography
TS	thymidylate synthase
UV	ultraviolet

vol.	volume
W	watt
wt.	weight
$[M^+]$	molecular ion
$[M\text{-}H^+]$	protonated molecular ion
δ	chemical shift
℃	degree celsius
µL	microliter
%	percent

CHAPTER I

INTRODUCTION

1.1 Pyrimidine

Pyrimidines are heterocyclic aromatic compounds containing two nitrogen atoms at positions 1 and 3 of its six-membered rings. Several pyrimidine derivatives including uracil, thymine and cytosine are biologically important as nucleotide bases in nucleic acids (Figure 1.1).

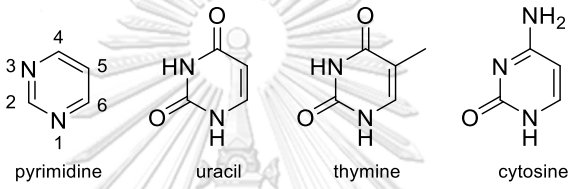


Figure 1.1 Structure of pyrimidine and pyrimidine nitrogenous bases.

In addition, pyrimidine ring is found in many natural products such as vitamin B_1 (thiamine) and alloxan. It is also found in many synthetic biologically active compounds such as barbituric acid and barbital which are used as hypnotics,[1] bacimethrin and sparsomycin which are used as antibiotics[2-3] (Figure 1.2).

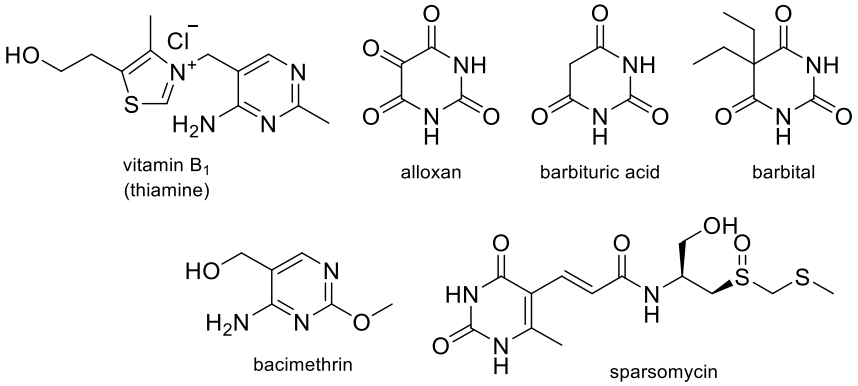


Figure 1.2 Natural and synthetic pyrimidine-containing compounds.

Pyrimidine-containing compounds have been of considerable interest due to theirs biologically effects, therapeutic potential and flexibility to derivatize at various positions. They are also well-known as biologically active compound possessing broad spectrum of pharmacological effects. Various analogs of pyrimidines have been reported with antibacterial,[4-6] antiallergic,[7] antitumor,[8-9] antifungal,[10] antipyretic,[11-12] anti-inflammatory,[12-14] antitubercular,[15] and antimalarial[16] activities. In addition to this, pyrimidine derivatives have been found to act as calcium channel blockers[17] and also possess potential central nervous system (CNS) depressant activities.[18-19]

1.2 Pyrimidine-based Antimalarial

Malaria is an infection caused by *Plasmodium* parasites which are transmitted to human through the bite of infected female *Anopheles* mosquitoes. There are only six *Plasmodium* species that are known to infect human. The most ferocious and predominant species that causes the infection is *Plasmodium falciparum*, with more than 200 million cases and 445,000 death in 2016.[20]

Plasmodium falciparum dihydrofolate reductase (DHFR) is an important target of antimalarial drug. DHFR is an enzyme which catalyzes the reduction of dihydrofolate to tetrahydrofolate. The *de novo* biosynthesis of folates which is catalyzed by DHFR in the presence of thymidylate synthase (TS) is indispensable in *Plasmodium* species as it is needed in DNA biosynthesis (Figure 1.3).[21] Inhibition of DHFR interferes with the DNA biosynthesis leading to the death of the *Plasmodium* parasites.

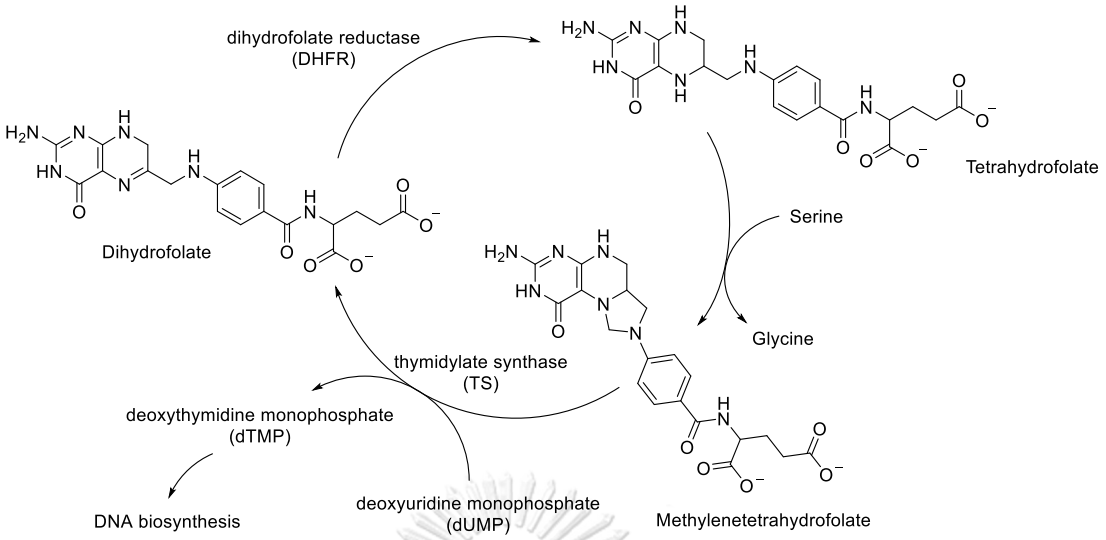


Figure 1.3 The role of dihydrofolate reductase (DHFR) in biotransformation of dUMP to dTMP.

Cycloguanil – a metabolite of the biguanidine proguanil (Figure 1.4)[22] - was identified as a potent dihydrofolate reductase inhibitor. It has long been used clinically in the treatment of malarial infection. In 1951, Falco and co-workers reported a series of 2,4-diaminopyrimidines with antimalarial activity.[23] The structure-activity relationship study revealed that the activity was enhanced when the alkyl group was introduced at the 6-position of 2,4-diaminopyrimidine. The activity was also enhanced when the phenyl group at the 5-position of 2,4-diaminopyrimidine was substituted in the *para*-position by halogen or nitro groups. Pyrimethamine (PYR) (Figure 1.4) which is a derivative of 2,4-diaminopyrimidine containing *p*-chlorophenyl and ethyl groups at the 5- and 6-positions, respectively, was reported as a potent inhibitor that showed the highest inhibition against malarial DHFR. However, the clinical efficacy of both cycloguanil and pyrimethamine has been compromised due to the mutations in the *Plasmodium falciparum* DHFR gene which led to antifolate resistance.[24]

Due to the observed resistance, other cycloguanil derivatives were further developed as an antifolate-based antimalarial.[25-26] The triazine derivative, WR99210 (Figure 1.4), possessed high activity against both wild-type and PYR-resistant mutant

strains of *Plasmodium falciparum*. However, it was found to possess severe gastrointestinal toxicity and had low bioavailability.

In 2012, Yuthavong and co-workers reported a pyrimidine derivative P65 carrying a flexible alkoxy side chain similar to WR99210 (Figure 1.4) as a new DHFR inhibitor.[27] This compound showed potency against *Plasmodium falciparum* DHFR and showed high bioavailability in rats. The enhanced bioavailability was thought to be due to the lower pK_a (basicity) of the diaminopyrimidine compared to the dihydrotriazine ring of cycloguanil. Further structural optimization leads to P218 (Figure 1.4) – a potent DHFR inhibitor with capability of binding to both wild-type and quadruple mutant *Plasmodium falciparum* DHFRs. It showed good selectivity between malarial and human enzymes as it did not efficiently bind to human DHFR. P218 is currently under phase I clinical trials.[28]

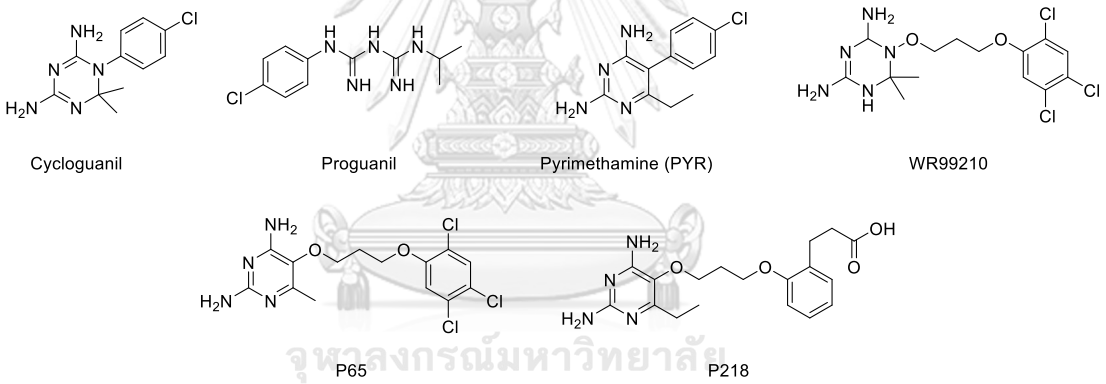


Figure 1.4 Pyrimidine-based malarial dihydrofolate reductase (DHFR) inhibitors.

Additionally, some related derivatives of 2,4-diaminopyrimidine were found to possess inhibition activity against *leishmanial* DHFR. These compounds also consisted of the 2,4-diaminopyrimidine core structure with 5-alkoxy and 6-alkyl substituents similar to P65 and P28 (Figure 1.5).[29]

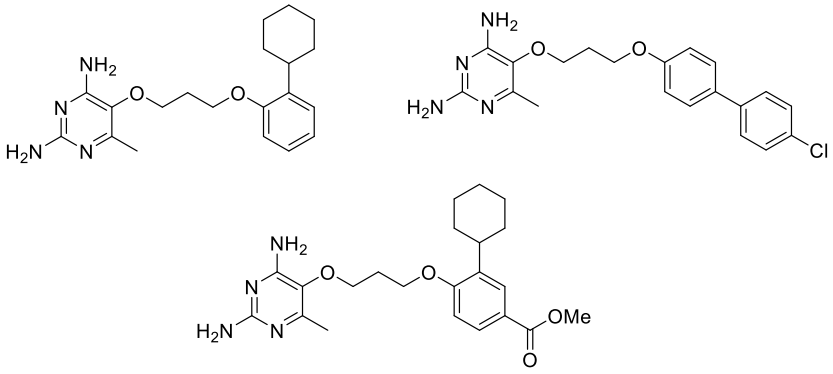


Figure 1.5 A series of 2,4-diaminopyrimidine derivatives as *leishmanial* DHFR inhibitors.

1.3 Synthesis of 2,4-Diaminopyrimidine-based DHFR Inhibitors

2,4-Diaminopyrimidine-based DHFR inhibitors consisted of the 2,4-diaminopyrimidine core structure, an alkyl group at the position 6 and an aryl substituted alkoxy side-chain at the position 5. 2,4-Diaminopyrimidines could be generally synthesized from the cyclo-condensation between guanidine and a 1,3-dicarbonyl compound followed by chlorination with phosphoryl chloride. The chloro could be converted to amino group by treatment with ammonia (Figure 1.6).[30-31]

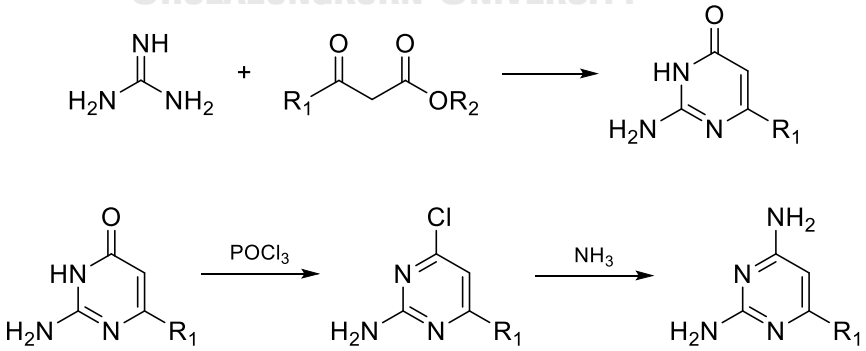


Figure 1.6 Synthesis of 2,4-diaminopyrimidine starting from guanidine and acetoacetate ester.

Preparation of DHFR inhibitors bearing an alkoxy substituent such as P65 or P218, it typically involved functionalization of 2,4-diamino-5-hydroxypyrimidines. The 5-hydroxypyrimidines could be obtained by direct C–H oxidation of 2,4-diaminopyrimidines, most frequently by the Boyland-Sims oxidation which employed persulfate as oxidant. This procedure typically gave low yields of the 5-hydroxylated products. However, other oxidation methods such as oxidation with organic peroxide or aromatic hydroxylation might be applied.

1.3.1 Boyland-Sims Oxidation of 2,4-Diaminopyrimidines

The Boyland-Sims oxidation is a reaction between an aromatic amine and persulfate anion in aqueous basic solvent to produce *ortho*-aminoaryl sulfate. The mechanism involved a nucleophilic attack of the amine on the peroxide oxygen to produce directly an intermediate aryl hydroxylamine–*O*–sulfonate after which rearranges to zwitterionic intermediate the *ortho* sulfate. The hydrolysis of *ortho* sulfate intermediate gives *ortho* hydroxy aryl amine as a product (Figure 1.7).[32]

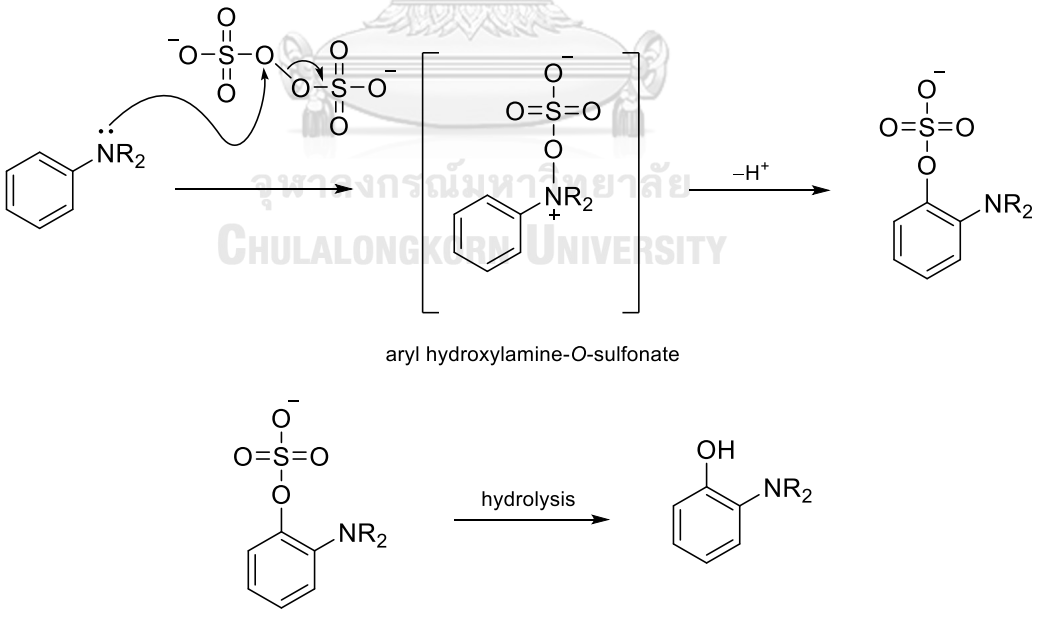


Figure 1.7 Reaction mechanism of Boyland-Sims oxidation.

The Boyland-Sims oxidation was reported to be compatible with several aromatic amine substrates. A significant disadvantage to the reaction is the low yield which is typically less than 50%, even though the peroxydisulfate anion is always completely consumed.[33-34]

The Boyland-Sims oxidation of 2,4-diaminopyrimidine was first attempted by Hull in 1956.[35] It was reported that unsubstituted 2,4-diaminopyrimidine was successfully oxidized to 2,4-diamino-5-hydroxypyrimidine in 47% overall yield using ammonium persulfate in an aqueous sodium hydroxide solution. Additionally, the derivative of 2,4-diaminopyrimidine with 6-methyl substituent was similarly oxidized in 58% overall yield under the same conditions (Figure 1.8).

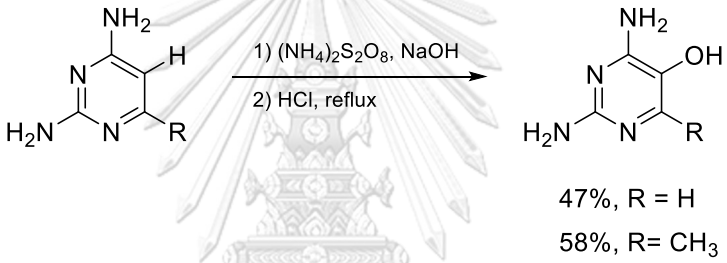


Figure 1.8 Oxidation of 2,4-diaminopyrimidines with ammonium persulfate.

1.3.2 Other C5-oxidations of 2,4-Diaminopyrimidine Derivatives

Some derivatives of 2,4-diaminopyrimidine have been reported to be successfully converted to 2,4-diamino-5-hydroxypyrimidines under alternative conditions. In 1975, McCall and Tenbrink reported the oxidation of 2,4-diamino-6-piperidinnopyrimidine using benzoyl peroxide. It was reported that the benzoate ester of 2,4-diamino-6-piperidinnopyrimidine was obtained in 48% and 21% yield in acetic acid and dichloromethane, respectively. Then, the ester was subsequently hydrolyzed with a base to afford 2,4-diamino-5-hydroxy-6-piperidinnopyrimidine in 60% yield (Figure 1.9).[36] They suggested that piperidine ring at the 6-position facilitated the C-5 oxidation since the electron density on the pyrimidine ring was increased by the presence of three strong electron-donating groups.

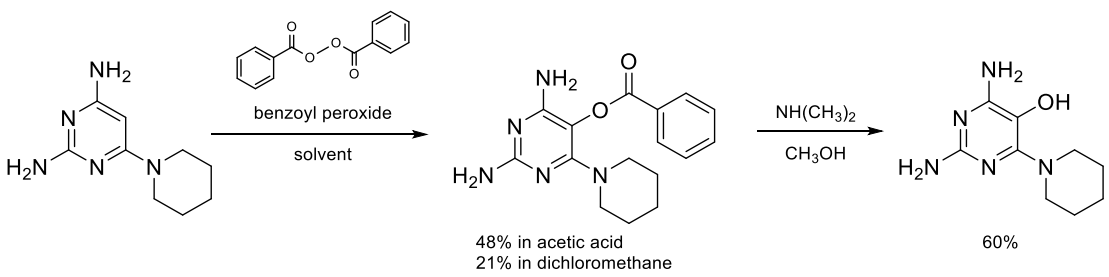


Figure 1.9 Oxidation of 2,4-diamino-6-piperidinnopyrimidine using benzoyl peroxide.

In 1994, Cabaj and co-workers proposed the preparation of pyrimidinyl 5-acetate ester under mild conditions, employing bromine-mediated nucleophilic addition-elimination in the presence of acetate salts.[37] The electron-rich pyrimidine with pyrrolidine and piperidine substituents at the positions 2, 4, and 6 gave 71% yield of the ester product, which was subsequently hydrolyzed to give 5-hydroxypyrimidine. However, the corresponding C-5 brominated pyrimidine was obtained in 81% instead under the same conditions when the pyrimidine was substituted with electron-withdrawal chloro group at the position 5 (Figure 1.10).

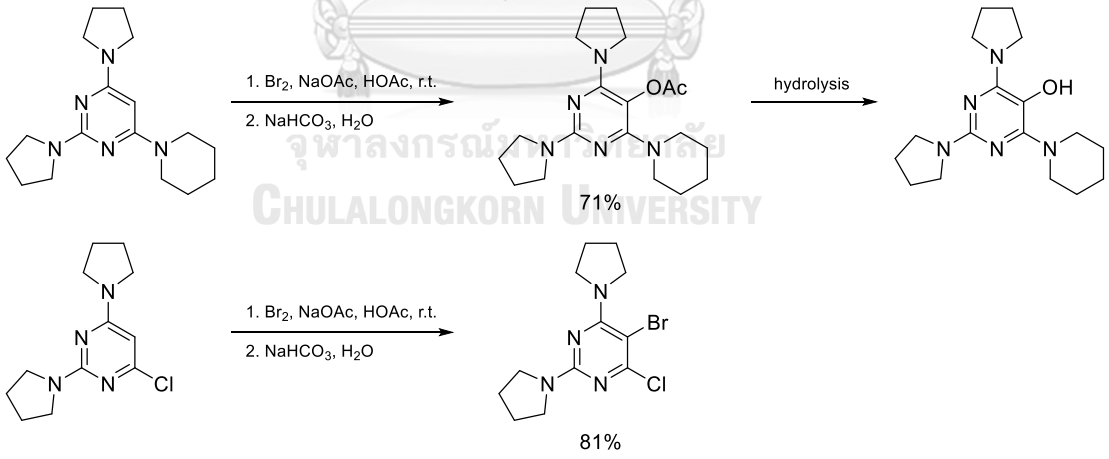


Figure 1.10 Bromine-mediated nucleophilic addition of 2,4-diaminopyrimidines.

1.3.3 Aromatic Hydroxylation

As the 2,4-diaminopyrimidines possess an aromaticity, direct hydroxylation might be applied in order to afford 2,4-diamino-5-hydroxypyrimidine. In 1975, Kovacic and co-workers propose an oxygenation of aromatic compounds such as benzene, chlorobenzene and anisole employing benzoyl peroxide and molecular iodine as oxidants.[38] The benzoate ester product was obtained in 25-87% along with iodinated side products (Figure 1.11).

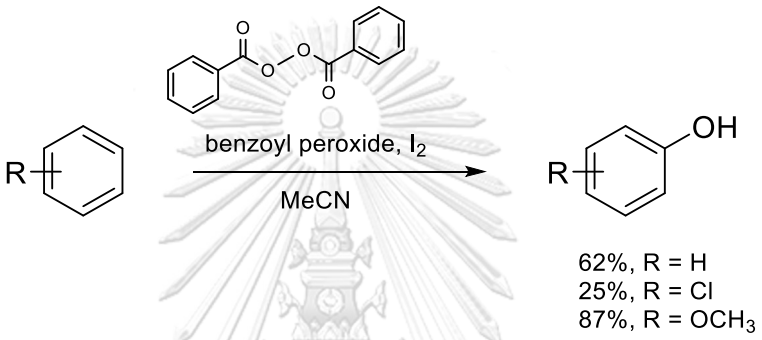


Figure 1.11 Aromatic oxygenation of aromatic compound using benzoyl peroxide and molecular iodine.

Recently in 2013, Yuan and co-workers proposed the direct aromatic hydroxylation using phthaloyl peroxide.[39] The aromatic C–H bond was oxidized directly through the reverse-rebound mechanism, leading to the hydroxylated products with excellent selectivity and no over-oxidation was observed (Figure 1.12). They also demonstrated that the method could be applied to various substituted aromatic compounds carrying a broad range of functional groups.

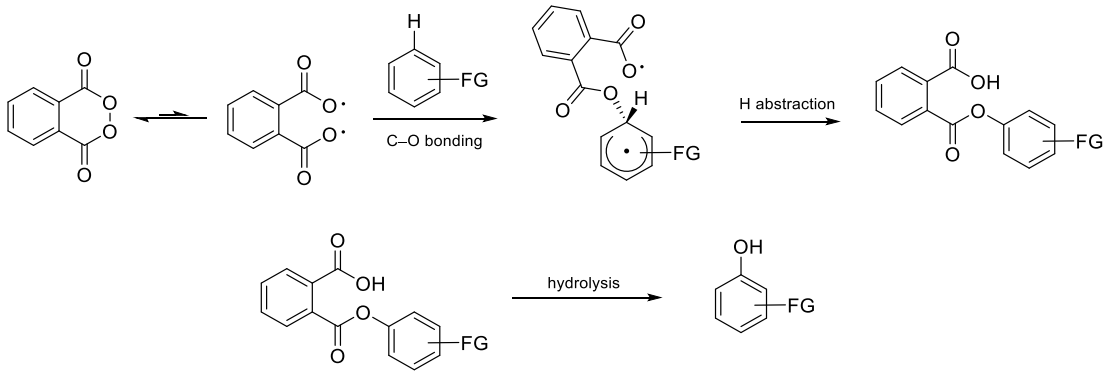


Figure 1.12 Direct aromatic hydroxylation using phthaloyl peroxide through the reverse-rebound mechanism.[39]

Several metal-catalyzed hydroxylations of aryl halide to afford hydroxylated aromatic compounds were also reported, but the halogenated aromatic substrates must be prepared as an intermediate before being converted to the hydroxylated products.[40-43] These conditions required ligands and some additives to perform the reaction along with transition metal salts as catalysts.

1.4 Objectives

2,4-Diamino-5-hydroxypyrimidine is an important precursor for the synthesis of 2,4-diaminopyrimidine-based dihydrofolate reductase (DHFR) inhibitors. The common route to prepare the 5-hydroxypyrimidine from 2,4-diaminopyrimidine is to directly oxidize at the C-5 position using persulfate oxidation (also known as Boyland-Sims oxidation) which usually gives low yields of the hydroxylated products. This research aimed to develop the the C-5 oxidation of 2,4-diaminopyrimidines further in order to improve the yield of 2,4-diamino-5-hydroxypyrimidines. The Boyland-Sims oxidation of 2,4-diaminopyrimidine will be optimized and applied to various 2,4-diaminopyrimidine substrates to explore the scope of the reaction. Alternative oxidation methods such as oxidation with organic peroxide or aromatic hydroxylation will also be explored. The success of this improved oxidation would be useful for development of novel 2,4-diaminopyrimidine antifolates with better activities and bioavailability in the future.

CHAPTER II

EXPERIMENTAL

2.1 Materials and Chemicals

All reactions were performed in oven-dried glassware. All reagent grade chemicals for the synthesis in this work were purchased from Acros, Merck, Sigma-Aldrich, TCI, or Fluorochem and were used without further purification. Laboratory grade organic solvents from RCI Labscan were used for column chromatography and thin-layer chromatography. Unless otherwise specified, analytical grade organic solvents from Burdick&Jackson and RCI Labscan were used for reaction set ups. The progress of the reactions was monitored by thin layer chromatography (TLC) performed on Merck D.C. silica gel 60 F_{254} 0.2 mm precoated aluminium sheets and visualized using UV light (254 nm) or potassium permanganate. Column chromatography was performed on Merck 70-230 mesh ASTM silica gel. Solvents for NMR experiments were purchased from Cambridge Isotope Laboratories or Euriso-top.

2.2 Instruments and Equipments

The weight of all chemical substances was determined on an AND GR-200 electrical balance. Evaporation of solvents was carried out on Büchi Rotavapor R-200 equipped with a Büchi Heating Bath B-490 and a Büchi Recirculating Chiller B-740.

Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker Avance 400 NMR spectrometer operating at 400 MHz for ^{1}H NMR experiments, 377 MHz for ^{19}F NMR experiments and 100 MHz for ^{13}C NMR experiments using deuterated dimethylsulfoxide (DMSO-d_6), deuterium oxide (D_2O), and deuterated chloroform ($CDCl_3$) as solvents. The chemical shift (δ) are reported in parts per million (ppm) relative to tetramethylsilane signal (δ_H = 0.00 ppm) employing residual protonated signal of deuterated solvent as a reference. Coupling constants (J) were reported in hertz (Hz). Multiplicities were abbreviated as followed: s = singlet, d =

doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet, and br = broad.

Mass spectra were obtained by Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry on a Bruker Daltonics Microflex Mass Spectrometer. The instrument was equipped with a nitrogen laser to desorb and ionize the samples. A stainless steel target was used as the substrate on which the samples were deposited. Samples were prepared as solutions in micromolar concentration in methanol, and α-cyano-4-hydroxycinnamic acid (CCA) was utilized as the matrix. Exact masses of some fluorinated compounds were elucidated by High Resolution Electrospray Ionization Quadrupole Time-of-Flight (ESI-QTOF) mass spectrometry operating on a Bruker MicrOTOF Q-II Mass Spectrometer.

2.3 Synthesis of 2-Amino-4-chloro-6-ethylpyrimidine

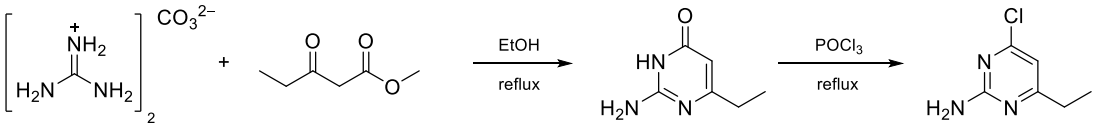


Figure 2.1 Synthesis of 2-amino-4-chloro-6-ethylpyrimidine.

Guanidine carbonate (20.0 mmol, 1.801 g) and methyl propionylacetate (40.0 mmol, 5.206 g) were dissolved in ethanol (20 mL). The mixture was refluxed for 48 hours. The white precipitate of 2-amino-6-ethyl-4(3*H*)-pyrimidinone was filtered with suction, dried and allowed to use in the next step without further purification. Phosphoryl chloride (8 mL) was added to the dried solid (5.455 g) and the reaction mixture was refluxed at 100°C until the starting material was completely consumed as monitored by TLC. After the reaction mixture was cooled down to room temperature, the solution was added dropwise to a cold saturated sodium hydrogen carbonate solution to destroy the excess $POCl_3$. The pale-yellow solid was filtered with suction and washed with cold water and allowed to dry, affording the title compound. (4.286 g, 76% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.15 (t, 3H), 2.50 (q, 2H), 6.56 (s, 1H), 6.98 (brs, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 12.3, 29.8, 106.9, 160.0, 163.4, 174.6. MS (MALDI-TOF): *m/z* calculated for $C_6H_8N_3Cl$ 157.041 [M^+], found 157.609. ^{1}H and ^{13}C NMR data are consistent with literature.[44]

2.4 Synthesis of 2,4-Diamino-6-ehthylpyrimidine (1a)

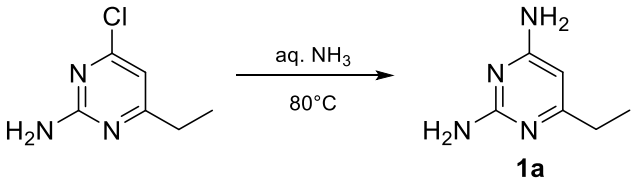


Figure 2.2 Synthesis of 2,4-diamino-6-ehthylpyrimidine (**1a**).

To a 50 mL stainless steel high pressure reaction vessel, 2-amino-4-chloro-6-ethylpyrimidine (30 mmol, 4.175 g) and aqueous ammonia solution (30% wt/vol., 20 mL) were added. The mixture was stirred at 80°C until the starting material was completely consumed. The ammonia was then removed under reduced pressure, and the solid was filtered and allowed to dry, affording compound **1a** as a pale-yellow solid (3.399 g, 82%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.15 (t, 3H), 2.43 (q, 2H), 5.79 (s, 1H), 6.96 (brs, 2H), 7.40 (brs, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 11.3, 25.0, 94.0, 156.0, 157.0, 165.2. MS (MALDI-TOF): *m/z* calculated for $C_6H_{10}N_4$ 138.090 [M^+], found 138.588. ^{1}H and ^{13}C NMR data are consistent with literature.[44]

2.5 Synthesis of 2-Amino-4-chloro-6-isopropylpyrimidine

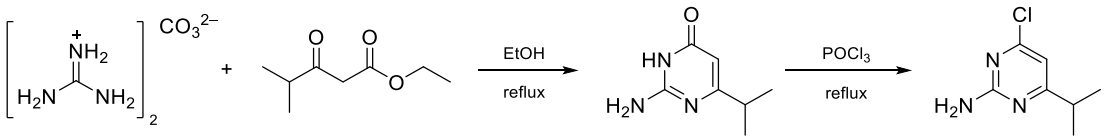
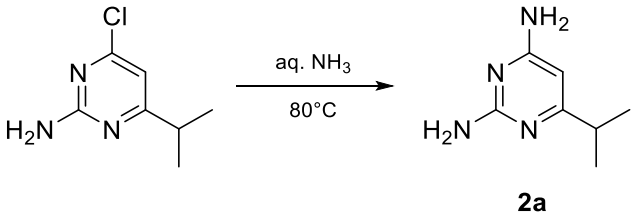


Figure 2.3 Synthesis of 2-amino-4-chloro-6-isopropylpyrimidine.

Guanidine carbonate (15.0 mmol, 1.351 g) and ethyl isobutyrylacetate (20.0 mmol, 3.164 g) were dissolved in ethanol (10 mL). The mixture was refluxed for 48 hours. The white precipitate of 2-amino-6-isopropyl-4(3*H*)-pyrimidinone was filtered with suction, dried and allowed to use in the next step without further purification. Phosphoryl chloride (5 mL) was added to the dried solid (1.446 g) and the reaction mixture was refluxed at 100°C until the starting material was completely consumed as monitored by TLC. After the reaction mixture was cooled down to room temperature, the solution was added dropwise to a cold saturated sodium hydrogen carbonate solution to destroy the excess $POCl_3$. The pale-yellow solid was filtered with suction and washed with cold water and allowed to dry, affording the title compound (0.841 g, 25% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.16 (d, 6H), 2.75 (m, 1H), 6.57 (s, 1H), 7.00 (brs, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.2, 35.0, 105.7, 160.2, 163.3, 178.3. MS (MALDI-TOF): *m/z* calculated for $C_7H_{10}N_3Cl$ 171.056 [M^+], found 171.587. ^{1}H and ^{13}C NMR data are consistent with literature.[30]

2.6 Synthesis of 2,4-Diamino-6-isopropylpyrimidine (2a)



2a

Figure 2.4 Synthesis of 2,4-diamino-6-isopropylpyrimidine (**2a**).

To a 50 mL stainless steel high pressure reaction vessels, 2-amino-4-chloro-6-isopropylpyrimidine (5.5 mmol, 0.841 g) and aqueous ammonia solution (30% wt/vol., 10 mL) were added. The mixture was stirred at 80°C until the starting material was completely consumed. Ammonia was then removed under reduced pressure, and the solid was filtered and allowed to dry, affording compound **2a** as a pale-yellow solid (0.574 g, 77%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.21 (d, 6H), 2.75 (m, 1H), 5.88 (s, 1H), 7.55 (brs, 2H), 7.95 (brs, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 20.2, 31.3, 92.5, 156.6, 161.5, 165.3. MS (MALDI-TOF): *m/z* calculated for $C_7H_{12}N_4$ 152.106 [M^+], found 152.588. ^{1}H and ^{13}C NMR data are consistent with literature.[30]

2.7 Synthesis of 2-Amino-4-chloro-6-trifluoromethylpyrimidine

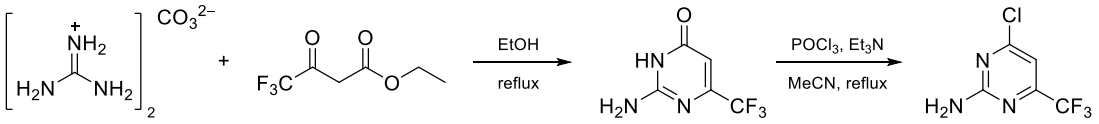


Figure 2.5 Synthesis of 2-amino-4-chloro-6-trifluoromethylpyrimidine.

Guanidine carbonate (24 mmol, 4.324 g) and ethyl-4,4,4-trifluoroacetoacetate (20 mmol, 3.682 g) were dissolved in ethanol (20 mL). The mixture was refluxed for 48 hours. The solvent was evaporated and ethanol (10 mL) was further added. The white solid was filtered off. The solution was then concentrated and purified by flash column chromatography on silica gel (50% methanol in ethyl acetate) to afford 2-amino-6-trifluoromethyl-4(3*H*)-pyrimidinone as a white solid (3.538 g). Acetonitrile (20 mL) was then added to dissolve the solid, followed by triethylamine (30 mmol, 3.421 g). Phosphoryl chloride (7 mL) was then added dropwise to the mixture. The reaction mixture was further refluxed until starting material was completely consumed monitored as by TLC. The solvent was removed under reduced pressure. The residue was gradually transferred into cold saturated sodium hydrogen carbonate solution to destroy the excess $POCl_3$. The yellow solid was filtered with suction and washed with cold water and allowed to dry, affording the title compound (3.859 g, 98% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.11 (s, 1H), 7.76 (brs, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 104.7 (q, $^3J_{C\text{-}F}$ = 2.9 Hz), 120.1 (q, $^1J_{C\text{-}F}$ = 276.2 Hz), 156.6 (q, $^2J_{C\text{-}F}$ = 34.9 Hz), 162.4, 163.4; ^{19}F NMR (377 MHz, DMSO-d_6) δ (ppm): −69.4. MS (MALDI-TOF): *m/z* calculated for $C_5H_3N_3ClF_3$ 196.997 [M^+], found 197.673. ^{1}H and ^{13}C NMR data are consistent with literature.[31]

2.8 Synthesis of 2,4-Diamino-6-trifluoromethylpyrimidine (4a)

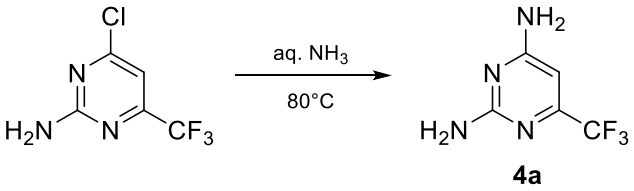


Figure 2.6 Synthesis of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**).

To a 50 mL glass pressure tube, 2-amino-4-chloro-6-trifluoromethylpyrimidine (19.5 mmol, 3.859 g) and ammonia solution (30% wt/vol., 20 mL) were added. The mixture was stirred at 80°C until the starting material was completely consumed. The excess ammonia was then removed under reduced pressure, and the residual solid was filtered, wash with water and allowed to dry, affording compound **4a** as a pale-yellow solid (3.079 g, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 6.05 (s, 1H), 6.43 (brs, 2H), 6.86 (brs, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 90.9 (q, $^{3}J_{C\text{-}F}$ = 3.4 Hz), 121.2 (q, $^{1}J_{C\text{-}F}$ = 274.5 Hz), 153.7 (q, $^{2}J_{C\text{-}F}$ = 32.8 Hz), 163.7, 165.0; ^{19}F NMR (377 MHz, DMSO-d_6) δ (ppm): –70.0. MS (MALDI-TOF): *m/z* calculated for $C_5H_5N_4F_3$ 178.047 [M^+], found 178.625. HRMS (ESI-QTOF): *m/z* calculated for $C_5H_5N_4F_3$ 179.0545 [M-H^+], found 179.0538.

2.9 Synthesis of 2,4-Diamino-6-ehthyl-5-pyrimidyl Hydrogen Sulfate (1b)

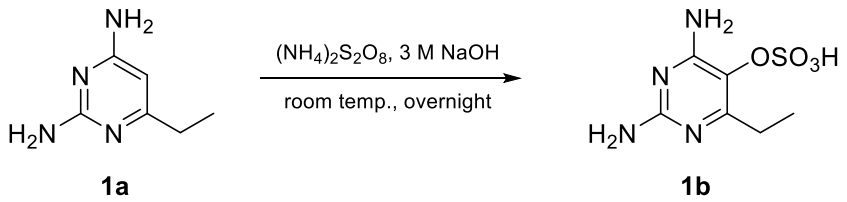
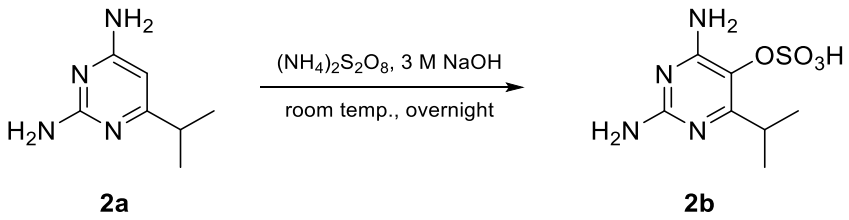


Figure 2.7 Synthesis of 2,4-diamino-6-ehthyl-5-pyrimidyl hydrogen sulfate (**1b**).

To a 25 mL round-bottom flask immersed in an ice bath, 2,4-diamino-6-ethylpyrimidine (**1a**, 3.0 mmol, 0.414 g) was dissolved in 3 M sodium hydroxide (7.50 mL). Ammonium persulfate (3.3 mmol, 0.753 g) was gradually added and the mixture was stirred at room temperature overnight. The reaction mixture was then cooled down to 0°C and acidified with concentrated hydrochloric acid. The yellow precipitate was filtered with suction and allowed to dry, affording compound **1b** (0.457 g, 65% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.15 (t, 3H), 2.62 (q, 2H), 7,19, (brs, 1H), 7.29 (brs, 2H), 8.34 (brs, 1H), 11,79 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 11.6, 20.6, 123.2, 148.8, 153.0, 161.5.

2.10 Synthesis of 2,4-Diamino-6-isopropyl-5-pyrimidinyl Hydrogen Sulfate (2b)

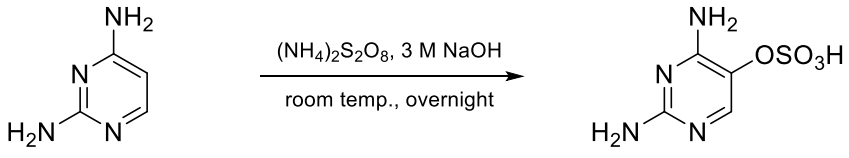


2a **2b**

Figure 2.8 Synthesis of 2,4-diamino-6-isopropyl-5-pyrimidinyl hydrogen sulfate (**2b**).

To a 25 mL round-bottom flask immersed in an ice bath, 2,4-diamino-6-isopropylpyrimidine (**2a**, 3.0 mmol, 0.456 g) was dissolved in 3 M sodium hydroxide (7.50 mL). Ammonium persulfate (3.3 mmol, 0.753 g) was gradually added and the mixture was stirred at room temperature overnight. The reaction mixture was then cooled down to 0°C and acidified with concentrated hydrochloric acid. The pale-yellow precipitate was filtered with suction and allowed to dry, affording compound **2b** (0.498 g, 67% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.18 (d, 6H), 3.43 (m, 1H), 7.23 (brs, 1H), 7.31 (brs, 2H), 8.39 (brs, 1H), 11.57 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 19.5, 25.9, 92.7, 122.2, 153.4, 161.8.

2.11 Synthesis of 2,4-diamino-5-pyrimidinyl Hydrogen Sulfate (3b)



3a **3b**

Figure 2.9 Synthesis of 2,4-diamino-5-pyrimidinyl hydrogen sulfate (**3b**).

To a 25 mL round-bottom flask immersed in an ice bath, 2,4-diaminopyrimidine (**3a**, 3.0 mmol, 0.330 g) was dissolved in 3 M sodium hydroxide (7.50 mL). Ammonium persulfate (3.3 mmol, 0.753 g) was gradually added and the mixture was stirred at room temperature overnight. The reaction mixture was then cooled down to 0°C and acidified with concentrated hydrochloric acid. The pale-yellow precipitate was filtered with suction and allowed to dry, affording compound **3b** (0.357 g, 58% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.47 (brs, 2H), 7.65 (brs, 1H), 7.67 (s, 1H), 8.38 (brs, 1H), 11.44 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 126.2, 130.6, 162.6, 160.7.

2.12 Synthesis of 2,4-Diamino-5-hydroxy-6-ethylpyrimidine (1c)

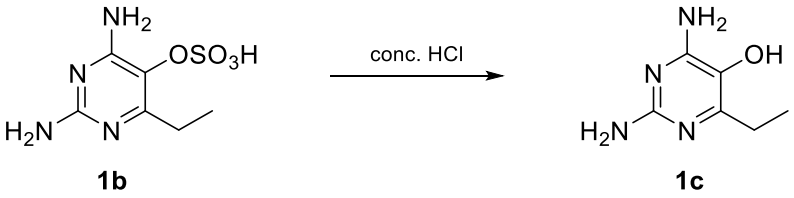


Figure 2.10 Synthesis of 2,4-diamino-5-hydroxy-6-ethylpyrimidine (**1c**).

To a 10 mL round-bottom flask, 2,4-diamino-6-ethyl-5-pyrimidinyl hydrogen sulfate (**1b**, 0.457 g, 1.95 mmol) was dissolved in concentrated hydrochloric acid (5.0 mL). The reaction mixture was refluxed at 100°C for 1 hour. The mixture was neutralized with sodium hydrogen carbonate and a few drops of ammonia solution until pH 7. The brown precipitate was filtered and allow to dry, affording compound **1c** (0.243 g, 81% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.11 (t, 3H), 2.48 (q, 2H), 6.21 (brs, 2H), 6.92 (brs, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 12.1, 22.0, 125.3, 147.3, 154.7, 158.8. MS (MALDI-TOF): *m/z* calculated for $C_6H_{10}N_4O$ 154.086 [M^+], found 154.594.

2.13 Synthesis of 2,4-Diamino-5-hydroxy-6-isopropylpyrimidine (2c)

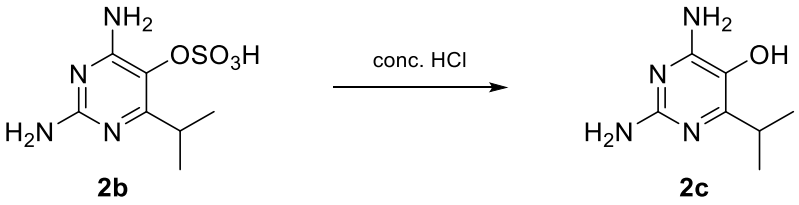


Figure 2.11 Synthesis of 2,4-diamino-5-hydroxy-6-isopropylpyrimidine (**2c**).

To a 10 mL round-bottom flask, 2,4-diamino-6-isopropyl-5-pyrimidinyl hydrogen sulfate (**2b**, 0.498 g, 2.0 mmol) was dissolved in concentrated hydrochloric acid (5.0 mL). The reaction mixture was refluxed at 100°C for 1 hour. The mixture was neutralized with sodium hydrogen carbonate and a few drops of ammonia solution until pH 7. The brown precipitate was filtered and allow to dry, affording compound **2c** (0.291 g, 86% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.14 (d, 6H), 3.28 (m, 1H), 6.62 (br, 2H), 7.22 (br, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 20.2, 26.1, 124.1, 154.5, 159.5. MS (MALDI-TOF): *m/z* calculated for $C_7H_{12}N_4O$ 168.101 [M^+], found 168.627.

2.14 Synthesis of 2,4-Diamino-5-hydroxypyrimidine (3c)

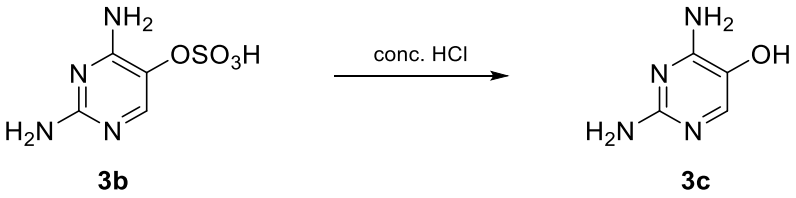


Figure 2.12 Synthesis of 2,4-diamino-5-hydroxypyrimidine (**3c**).

To a 10 mL round-bottom flask, 2,4-diamino-5-pyrimidinyl hydrogen sulfate (**3b**, 0.498 g, 2.0 mmol) was dissolved in concentrated hydrochloric acid (5.0 mL). The reaction mixture was refluxed at 100°C for 1 hour. The mixture was neutralized with sodium hydrogen carbonate and a few drops of ammonia solution until pH 7. The brown precipitate was filtered and allow to dry, affording compound **3c** (0.163 g, 75% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.09 (s, 2H), 7.26 (s, 1H), 7.85 (brs, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 122.1, 129.9, 152.4, 159.6. MS (MALDI-TOF): *m/z* calculated for $C_4H_6N_4O$ 126.054 [M^+], found 126.461.

2.15 General Procedure for Boyland-Sims Oxidation of 2,4-Diamino-6-ethylpyrimidine (1a)

To a round-bottom flask containing 2,4-diamino-6-ethylpyrimidine (**1a**, 13.8 mg, 0.10 mmol) and the specified amount of ammonium persulfate, solvents were added. The mixture was stirred at room temperature overnight. After solvent removal, 1,3,5-trimethoxybenzene (5.5 mg, 0.03 mmol) was added to the residue as an internal standard. The residue was dissolved in DMSO-d_6, and the yields of the 2,4-diamino-6-ethyl-5-pyrimidinyl hydrogen sulfate (**1b**) were determined by ^{1}H NMR. The isolated pyrimidinyl hydrogen sulfate (**1b**, 23.4 mg, 0.10 mmol) was dissolved in concentrated hydrochloric acid (300 µL) in a round-bottom flask to perform hydrolysis reaction. The mixture was refluxed at 100°C for 1 hour. After solvent removal, 1,3,5-trimethoxybenzene (5.5 mg, 0.03 mmol) was added to the residue as an internal standard. The residue was dissolved in DMSO-d_6, and the yields of the 2,4-diamino-5-hydroxy-6-ethylpyrimidine (**1c**) were determined by ^{1}H NMR.

2.16 Synthesis of Phthaloyl Peroxide

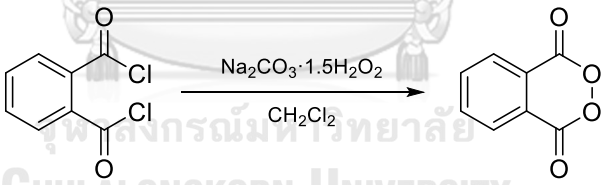


Figure 2.13 Synthesis of phthaloyl peroxide.

To a solution of phthaloyl dichloride (1.015 g, 5 mmol) in dichloromethane (15 mL), sodium percarbonate (1.178 g, 7.5 mmol) was added. The mixture was stirred vigorously at room temperature for 3 hours. The reaction mixture was filtered through Celite and concentrated to afford the title compound as a white solid (0.343 g, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.03 (dd, J = 5.7, 3.3 Hz, 2H), 8.29 (dd, J = 5.7, 3.3 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 123.7, 130.2, 136.4, 162.0. ^{1}H and ^{13}C NMR data are consistent with literature.[45]

2.17 Synthesis of *p*-Nitrobenzoyl Peroxide

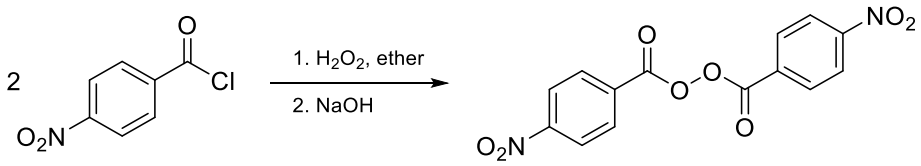


Figure 2.14 Synthesis of *p*-nitrobenzoyl peroxide.

To a cold solution of *p*-nitrobenzoyl chloride (1.8556 g, 10 mmol) in diethyl ether, hydrogen peroxide (665 µL, 30% wt. in H_2O, 5.85 mmol) was added dropwise over 10 minutes, followed by 5 M NaOH (5 mL) over 20 minutes. The white precipitate was filtered with suction and washed with water. The solid was recrystallized from hot toluene, affording the title compound as a pale-yellow solid (1.362 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.28 (d, *J* = 8.7 Hz, 2H), 8.40 (d, *J* = 8.7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 124.1, 130.6, 131.1, 151.4, 161.1. ^{1}H and ^{13}C NMR data are consistent with literature.[46]

2.18 Synthesis of 2,4-Diamino-6-trifluoromethylpyrimidine-3-*N*-oxide (4d)

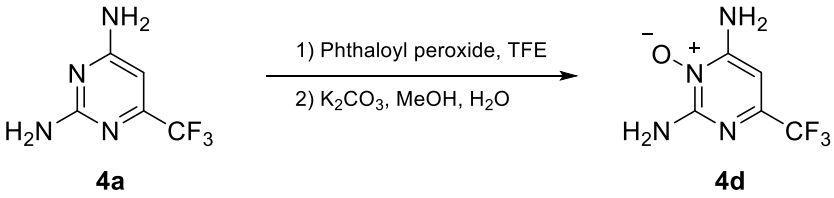


Figure 2.15 Synthesis of 2,4-diamino-6-trifluoromethylpyrimidine-3-*N*-oxide (**4d**).

A suspension of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**, 0.249 g. 1.4 mmol) and phthaloyl peroxide (0.345 g, 2.1 mmol) in trifluoroethanol (2 mL) was heated to 50°C and stirred under N_2 atmosphere for 8 hours. After the solvent was removed under reduced pressure, the residue was dissolved in methanol solution (5.5 mL, 75% vol. in water), follow by addition of potassium carbonate (0.774 g, 5.6 mmol). The reaction mixture was stirred at room temperature for 12 hours. The mixture was extracted with ethyl acetate (3×10 mL). The organic layer was washed with brine and dried over magnesium sulfate. After the solvent was removed, the crude mixture was purified by column chromatography using 10% methanol in dichloromethane as eluent, affording compound **4d** as a pale-yellow solid (0.124 g, 46%, R_f = 0.21, 5% methanol in dichloromethane). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 6.50 (s, 1H), 7.74 (br, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 91.1 (q, $^3J_{C\text{-}F}$ = 3.3 Hz), 121.0 (q, $^1J_{C\text{-}F}$ = 273.9 Hz), 139.8 (q, $^2J_{C\text{-}F}$ = 35.2 Hz), 152.5, 153.9; ^{19}F NMR (377 MHz, DMSO-d_6) δ (ppm): –68.7. HRMS (ESI-QTOF): *m/z* calculated for $C_5H_6N_4OF_3$ 195.0488 [M-H$^+$], found 195.0486.

2.19 Synthesis of 2,4-Diamino-5-chloro-6-trifluoromethylpyrimidine (4e)

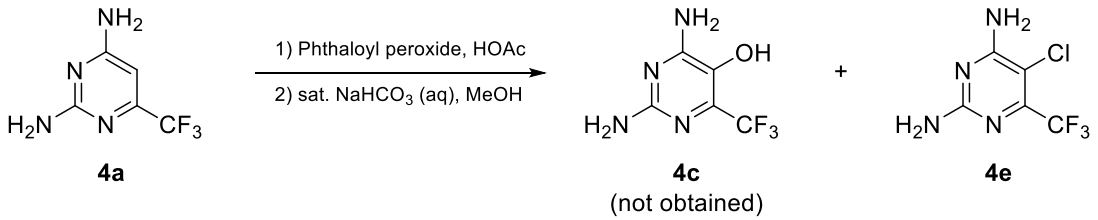


Figure 2.16 Synthesis of 2,4-diamino-5-chloro-6-trifluoromethylpyrimidine (**4e**).

A suspension of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**, 0.365 g. 2.0 mmol) and phthaloyl peroxide (0.492 g, 3.0 mmol) in acetic acid (6 mL) was heated to 50℃ and stirred for 8 hours. After acetic acid was removed under reduced pressure, methanol (6 mL) and saturated solution of sodium hydrogen carbonate (2 mL) were added. The reaction mixture was stirred further for 12 hours. The mixture was extracted with ethyl acetate (3×15 mL). The organic layer was dried over magnesium sulfate. After solvent removal, the crude mixture was purified by column chromatography using 5% methanol in dichloromethane as eluent, resulting as a pale-yellow solid of by-product **4e** (0.070 g, 18%, R_f = 0.38, 5% methanol in dichloromethane) instead of the desired product **4c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 6.63 (s, 2H), 7.21 (br, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 97.6, 120.8 (q, $^1J_{C\text{-}F}$ = 277.6 Hz), 148.8 (q, $^2J_{C\text{-}F}$ = 32.4 Hz), 160.8, 161.4; ^{19}F NMR (377 MHz, DMSO-d_6) δ (ppm): –66.2. HRMS (ESI-QTOF): *m/z* calculated for $C_5H_5N_4ClF_3$ 213.0149 [M-H$^+$], found 213.0143.

2.20 Synthesis of 2,4-Diamino-5-bromo-6-trifluoromethylpyrimidine (4f)

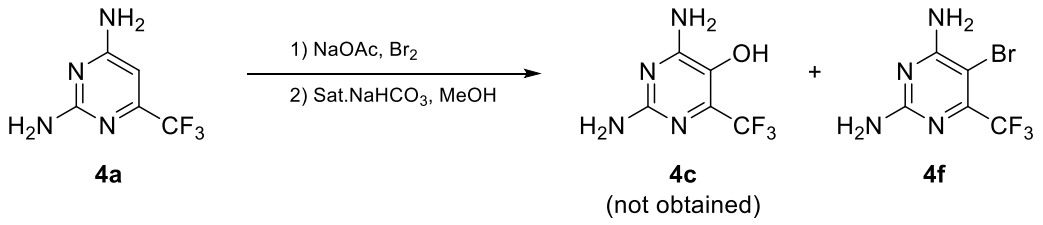


Figure 2.17 Synthesis of 2,4-diamino-5-bromo-6-trifluoromethylpyrimidine (**4f**).

To a flask containing 2,4-diamino-6-trifluoromethylpyrimidine (**4a**, 0.178 g, 1.0 mmol), dichloromethane (1 mL) was added, followed by sodium acetate (0.279 g, 3.4 mmol) and liquid bromine (52 µL, 1.0 mmol) at 0°C. The reaction mixture was slowly warmed to room temperature and stirred for 8 hours. After the solvent was removed, methanol (3 mL) and a saturated solution of sodium hydrogen carbonate (1 mL) were added. The reaction mixture was stirred further for 12 hours. The mixture was extracted with ethyl acetate (3×10 mL). The organic layer was dried over magnesium sulfate. After solvent removal, the crude mixture was purified by column chromatography using 5% methanol in dichloromethane as eluent, affording compound **4f** as a pale-yellow solid (0.276 g, 93%, R_f = 0.42, 5% methanol in dichloromethane) instead of the desired product **4c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 6.66 (s, 2H), 7.12 (br, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 85.5, 120.8 (q, $^1J_{C\text{-}F}$ = 279.4 Hz), 150.9 (q, $^2J_{C\text{-}F}$ = 32.6 Hz), 161.3, 162.2; ^{19}F NMR (377 MHz, DMSO-d_6) δ (ppm): –66.1. HRMS (ESI-QTOF): *m/z* calculated for $C_5H_5N_4BrF_3$ 256.9644 [M-H$^+$], found 256.9648.

2.21 Synthesis of 2,4-Diamino-5-iodo-6-trifluoromethylpyrimidine (4g)

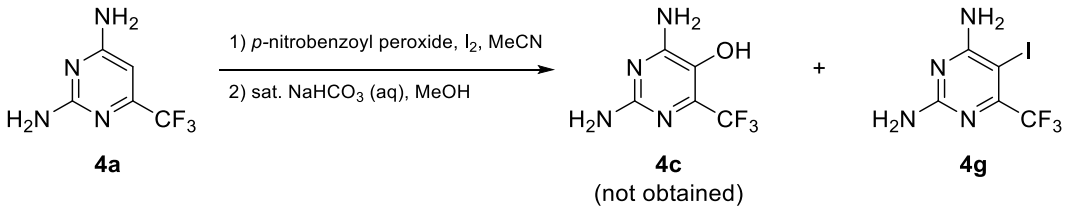


Figure 2.18 Synthesis of 2,4-diamino-5-iodo-6-trifluoromethylpyrimidine (**4g**).

A suspension of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**, 0.178 g, 1.0 mmol), *p*-nitrobenzoyl peroxide (0.498 g, 1.5 mmol), and iodine (0.076 g, 0.3 mmol) in acetonitrile (10 mL) was heated to 90°C and stirred for 8 hours. After acetronitrile was removed under reduced pressure, methanol (3 mL) and a saturated solution of sodium hydrogen carbonate (1 mL) were added. The reaction mixture was stirred further for 12 hours. The mixture was extracted with ethyl acetate (3×10 mL). The organic layer was dried over magnesium sulfate. After solvent removal, the crude mixture was purified by column chromatography using 5% methanol in dichloromethane as eluent, affording compound **4g** as a yellow solid (0.188 g, 62%, R_f = 0.49, 5% methanol in dichloromethane) instead of the desired product **4c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 6.59 (s, 2H), 6.97 (br, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 56.6, 121.0 (q, $^1J_{C\text{-}F}$ = 279.4 Hz), 154.9 (q, $^2J_{C\text{-}F}$ = 31.8 Hz), 162.2, 164.6; ^{19}F NMR (377 MHz, DMSO-d_6) δ (ppm): −65.8. HRMS (ESI-QTOF): *m/z* calculated for $C_5H_5N_4IF_3$ 304.9506 [M-H$^+$], found 304.9492.

2.22 General Procedure for Oxidation of 2,4-Diamino-6-trifluoromethylpyrimidine

To a vial containing 2,4-diamino-6-trifluoromethylpyrimidine (**4a**, 8.9 mg, 0.050 mmol), different oxidants, additives and solvents were added. The mixture was stirred at various temperatures and durations. After solvent removal, the residue was hydrolyzed with a mixture of methanol and saturated solution of sodium hydrogen carbonate. The mixture was stirred at room temperature for 8 hours. After solvent evaporation, trifluoroacetamide (5.7 mg, 1.0 equiv., 0.050 mmol) or trifluorotoluene (7.3 mg, 1.0 equiv., 0.050 mmol) were added to the crude mixture as internal standards followed by DMSO-d_6. The yields of the product(s) were determined by ^{19}F NMR.

2.23 General Procedure for Reduction of *N*-Oxides

To a 2 mL glass vial containing 2,4-diamino-6-trifluoromethylpyrimidine (**4a**, 8.9 mg, 0.050 mmol) and phthaloyl peroxide (24.6 mg, 0.150 mmol), aqueous acetic acid (HOAc:H_2O = 2:1 ,150 µL) was added. The reaction was stirred at room temperature for 8 hours. After solvent removal, the residual crude product was heated with phenylboronic acid (0.15 or 0.25 mmol) in dichloroethane (150 µL) or zinc dust (0.10 mmol), and ammonium formate (0.15 mmol) in methanol (150 µL) for 8 hours. After the solvent was evaporated, trifluorotoluene (7.3 mg, 1.0 equiv., 0.050 mmol) was added to the residual crude as internal standard followed by DMSO-d_6. The yields of the product(s) were determined by ^{19}F NMR.

2.24 General Procedure for Reaction of Halogenated 2,4-Diamino-6-trifluoromethylpydimidine with Tetrabutylammonium Hydroxide

To a solution of 2,4-diamino-5-chloro-6-trifluoromethylpydimidine (**4e**, 10.6 mg, 0.05 mmol), 2,4-diamino-5-bromo-6-trifluoromethylpydimidine (**4f**, 12.9 mg, 0.05 mmol), or 2,4-diamino-5-iodo-6-trifluoromethylpydimidine (**4g**, 15.2 mg, 0.05 mmol) in dimethylformamide (150 µL), aqueous tetrabutylammonium hydroxide solution (97 µL, 0.15 mmol) was added. The reaction mixture was heated to 150°C for 8 hours. After the solvent was evaporated, trifluorotoluene (7.3 mg, 1.0 equiv., 0.050 mmol) was added to the residual crude as internal standard followed by DMSO-d_6. The yields of the product(s) were determined by ^{19}F NMR.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Boyland-Sims Oxidation of 2,4-Diaminopyrimidines

Boyland-Sims oxidation of 2,4-diaminopyrimidines consists of two steps. Firstly, the pyrimidine substrate reacts with a persulfate anion under basic conditions to generate a pyrimidinyl hydrogen sulfate intermediate. The hydrogen sulfate group will be converted to a hydroxy group by acid hydrolysis (Figure 3.1).

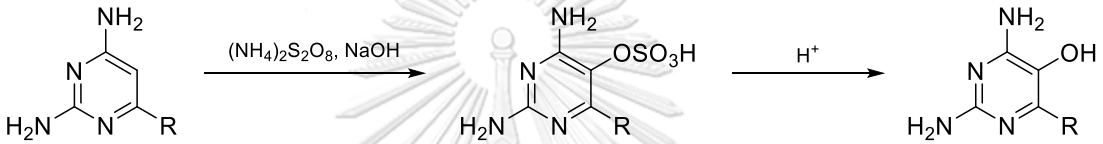


Figure 3.1 The two steps of Boyland-Sims oxidation of 2,4-diaminopyrimidines.

To study the Boyland-Sims oxidation of 2,4-diaminopyrimidines, 2,4-diamino-6-ethylpyrimidine (**1a**) was chosen as a model substrate. The model compound was successfully synthesized in three steps starting from guanidine carbonate and methyl propionylacetate using the reported conditions from literature[30] and was obtained in 62% overall yield. The white solid initially obtained from cyclo-condensation between the two starting materials was the corresponding 2-amino-6-ethyl-4(3*H*)-pyrimidinone. The solid was converted to the corresponding 4-chlorinated 2-aminopyrimidine intermediate by treating with phosphoryl chloride ($POCl_3$). Then the chloro group was converted to an amino group by heating with aqueous ammonia solution under high pressure, resulting in 2,4-diamino-6-ethylpyrimidine (**1a**) as a pale-yellow solid (Figure 3.2).

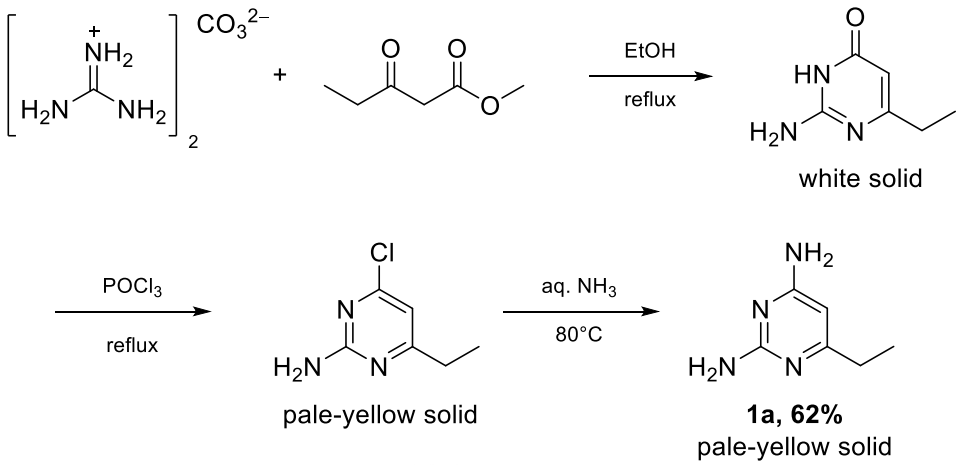


Figure 3.2 Synthetic route of 2,4-diamino-6-ethylpyrimidine (**1a**).

To investigate the parameters that affect the Boyland-Sims oxidation and to optimize the conditions, the reactions were performed at 0.10 mmol scale of 2,4-diamino-6-ethylpyrimidine (**1a**) with various parameters including: equivalents of ammonium persulfate, types and concentrations of base, and volumes of solvents. All reactions were performed overnight (ca 15 hours) at room temperature (ca 30°C). Then, the yields of the pyrimidinyl hydrogen sulfate were calculated using ^{1}H NMR spectroscopy compared to an internal standard (1,3,5-trimethoxybenzene, 0.03 mmol) added to the residue after solvent removal prior to the addition of DMSO-d_6. The signals at 3.70 and 6.09 ppm from the methoxy group and aromatic protons of the internal standard were used as references. The yield was calculated by integrating the signal at 1.15 ppm from the methyl group of 2,4-diamino-6-ethyl-5-pyrimidinyl hydrogen sulfate (**1b**) (Figure 3.3a). The authentic compound **1b** was spiked into the solution to confirm the identity of the desired product (Figure 3.3b).

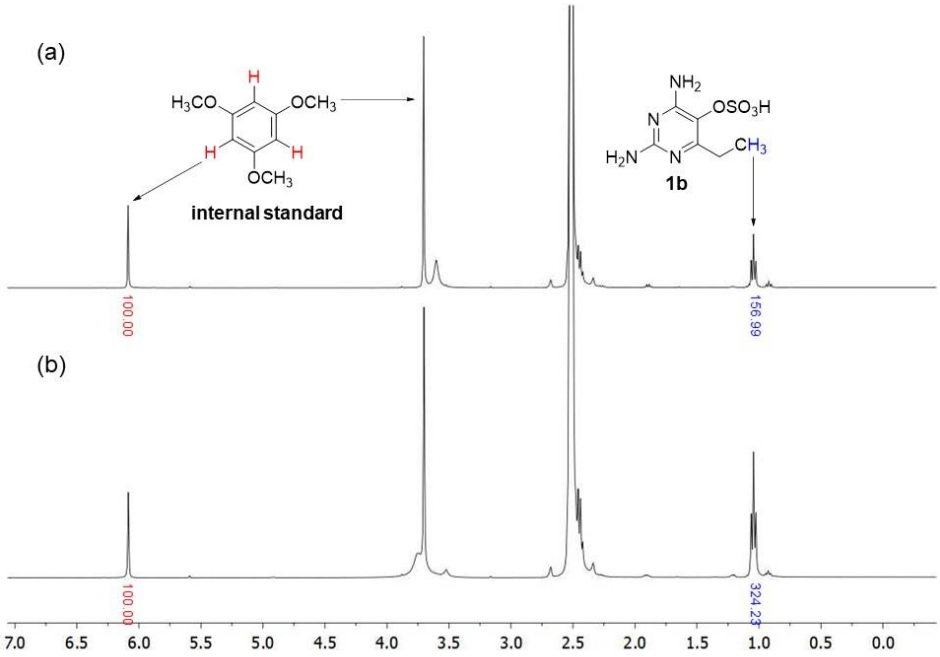


Figure 3.3 ^{1}H NMR spectra of the crude reaction mixture and 1,3,5-trimethoxybenzene as an internal standard before (a) and after (b) spiking of 2,4-diamino-6-ethyl-5-pyrimidinyl hydrogen sulfate (**1b**).

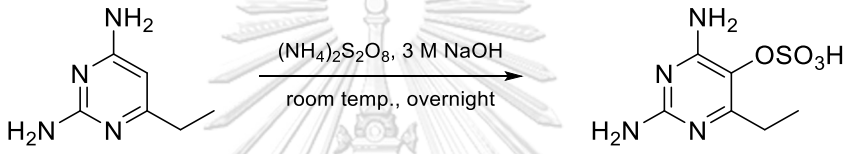
3.1.1 Effect of Equivalent of Ammonium Persulfate

The model compound, 2,4-diamino-6-ethylpyrimidine (**1a**), was allowed to react with various equivalents of ammonium persulfate in 3 M sodium hydroxide solution as summarized in Table 3.1. The NMR yield of 2,4-diamino-6-ethyl-5-pyrimidinyl hydrogen sulfate (**1b**) from the previously reported conditions[35] using 1.5 equivalents of ammonium persulfate in 300 µL of 3 M NaOH was 35% as determined based on the internal standard (entry 3). Too high amounts of the oxidant negatively affected the yield of the product. When the amount of ammonium persulfate was increased to 2.0 and 3.0 equivalents, the yield of the pyrimidinyl hydrogen sulfate **1b** significantly dropped to 25% and 10%, respectively (entries 4-5). This might be due to the formation of *N*-oxide species which could not dissolve in deuterated dimethyl sulfoxide. The *N*-oxide species could possibly be generated by the attack of nitrogen atom on the pyrimidine ring to the oxygen atom of persulfate anion. Additionally, the

N-oxide species are often found in the oxidation of pyrimidines using organic peroxides as oxidants.[47]

On the other hand, lowering the amount of ammonium persulfate to 1.1 equivalents raised the yield to 49% (entry 2). However, when the amount of the oxidant was further reduced to 1.0 equivalent, the yield of the product was slightly decreased (entry 1).

Table 3.1 Boyland-Sims oxidation of 2,4-diamino-6-ethylpyrimidine (**1a**) with various equivalents of ammonium persulfate.



1a **1b**

Entry	equiv. of $(NH_4)_2S_2O_8$	Concentration of base (M)	Volume of base (μL)	Conversion (%)[a]	Yield (%)[a]
1	1.0	3	300	100	45
2	1.1	3	300	100	49
3	1.5	3	300	100	35
4	2.0	3	300	100	25
5	3.0	3	300	100	10

[a]Yields and conversions were determined by ^{1}H NMR spectroscopy with 1,3,5-trimethoxybenzene as internal standard.

3.1.2 Effect of Concentration and Type of Base

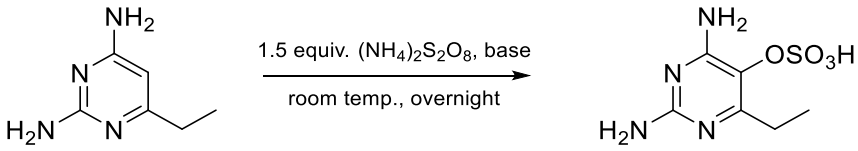
The reactions of the model compound **1a** with 1.5 equivalents of ammonium persulfate were performed in various volumes of solvent and concentrations of sodium hydroxide solution as summarized in Table 3.2. Lowering the volume of solvent did not significantly affect the yields of the pyrimidinyl hydrogen sulfate product **1b** (entries 1-3) comparing to the reaction under standard conditions (entry 4). Increasing volume of solvent might improve the solubility of the pyrimidine

substrate and led to higher yield of the desired product (entries 5-6). When the concentration of sodium hydroxide was reduced to 2 M, the substrate might be more soluble. The yield was higher than the reaction performed under standard conditions but not very significant (entry 7). However, the higher concentration of sodium hydroxide decreased solubility of the substrate. Even though the starting material was not completely consumed, the yield of the desired product was increased to 45% (entry 8).

In addition, the type of base was also varied. With the same concentration, reaction in lithium hydroxide solution, a lower yield of the pyrimidinyl hydrogen sulfate **1b** was obtained compared to the reaction in sodium hydroxide solution (entry 9). The yield of the desired product sharply increased to 47% when potassium hydroxide was used, but the substrate was poorly soluble in aqueous potassium hydroxide solution and led to incomplete consumption of the starting material (entry 10). The yield of the product significantly dropped to 22% when the reaction was performed in higher concentration of potassium hydroxide (entry 11).

The reactions were also performed with 1.1 equivalents of ammonium persulfate as this amount of the oxidant gave the highest yield of the desired product. The reactions gave lower yields of the pyrimidinyl hydrogen sulfate **1b** compared with the reaction under standard conditions since the high concentration of bases decreased the solubility of the starting material. The yields of the product were observed at 31% in 5 M sodium hydroxide solution and 15% in 5 M potassium hydroxide solution. The starting material was not completely consumed in both reactions (entries 12-13).

Table 3.2 Variation of concentrations and bases for Boyland-Sims oxidation of 2,4-diamino-6-ethylpyrimidine (**1a**).



1a **1b**

Entry	Concentration of base (M)	Volume of base (μL)	Base	Conversion (%)[a]	Yield (%)[a]
1	3	100	NaOH	100	36
2	3	150	NaOH	100	36
3	3	200	NaOH	100	37
4	3	300	NaOH	100	35
5	3	400	NaOH	100	40
6	3	500	NaOH	100	42
7	2	300	NaOH	100	40
8	5	300	NaOH	96	45
9	3	300	LiOH	100	32
10	3	300	KOH	89	47
11	5	300	KOH	72	22
12[b]	5	300	NaOH	85	31
13[b]	5	300	KOH	65	15

[a]Yields and conversions were determined by ^{1}H NMR spectroscopy with 1,3,5-trimethoxybenzene as internal standard. [b]1.1 equivalents of ammonium persulfate.

3.1.3 Hydrolysis of 2,4-Diamino-6-ethyl-5-pyrimidinyl Hydrogen Sulfate

The hydrogen sulfate intermediate, 2,4-diamino-6-ethyl-5-pyrimidinyl hydrogen sulfate (**1b**), was refluxed with concentrated hydrochloric acid for 1 hour to afford 2,4-diamino-5-hydroxy-6-ethylpyrimidine (**1c**). The reaction was performed at 0.10 mmol scale of the starting material. The yield of the final hydroxypyrimidine product **1c** was determined by ^{1}H NMR spectroscopy using 1,3,5-trimethoxybenzene

as an internal standard. The NMR yield of compound **1c** was 96% and the starting material **1b** was completely consumed (Figure 3.4), indicating a practically quantitative hydrolysis reaction.

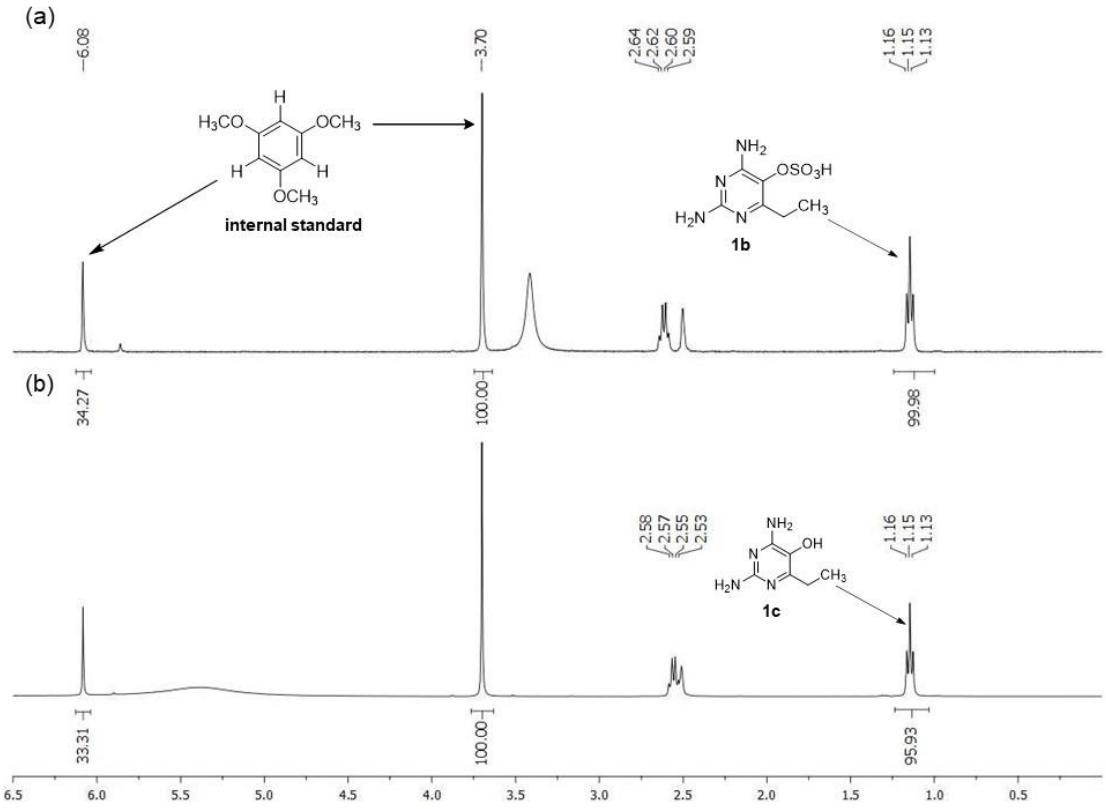


Figure 3.4 ^{1}H NMR spectra of (a) the reaction mixture of the pyrimidinyl hydrogen sulfate **1b** before hydrolysis and (b) the hydroxypyrimidine **1c** from hydrolysis and 1,3,5-trimethoxybenzene as an internal standard.

The hydrolysis process was also reproduced at preparative scale (1.0 mmol) of the starting material **1b**. The hydroxypyrimidine **1c** was obtained in 81% isolated yield as a brown solid. It should be noted that some loss of the product was inevitable due to partial solubility of **1c** in water.

3.1.4 Substrate Scope

The Boyland-Sims oxidation of 2,4-diamino-6-ethylpyrimidine (**1a**) was reproduced at a larger scale (3.0 mmol) employing the condition that gave the highest yield (Table 3.1 entry 2, 1.1 equivalents of ammonium persulfate in 3 M sodium hydroxide solution). The pyrimidinyl hydrogen sulfate **1b** was obtained in 65% as a pale-yellow solid. The hydrolysis of compound **1b** gave the product **1c** in 81%. The overall yield was 53% which was higher than the yield obtained from the reaction under the previously reported conditions (35%).[35]

The conditions were also applied to the derivatives of 2,4-diaminopyrimidines with different substituents at C-6 position. 2,4-Diaminopyrimidine (**3a**) and 2,4-diamino-6-chloropyrimidine (**5a**) were purchased from the commercial sources and were used without further purification. 2,4-Diamino-6-isopropylpyrimidine (**2a**) was synthesized in a similar fashion to 2,4-diamino-6-ethylpyrimidine (**1a**) starting from guanidine carbonate and ethyl isobutyrylacetate under the previously reported conditions.[30] The pale-yellow solid of the pyrimidine **2a** was obtained in 19% overall yield (Figure 3.5).

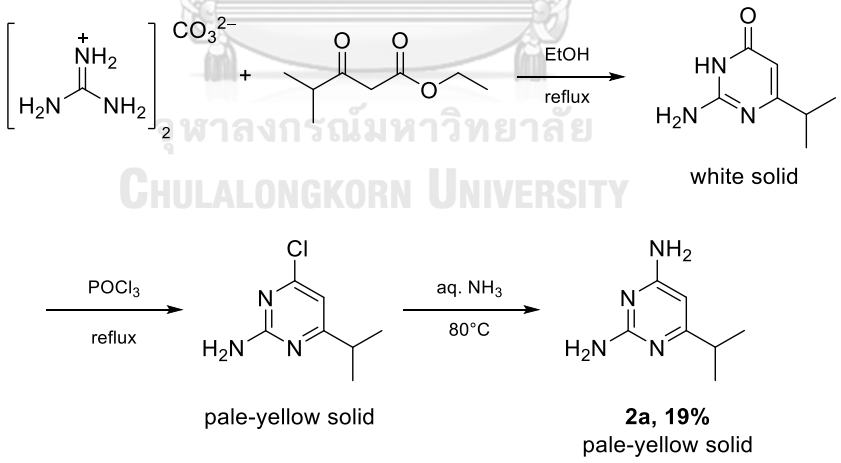


Figure 3.5 Synthetic route of 2,4-diamino-6-isopropylpyrimidine (**2a**).

2,4-Diamino-6-trifluoromethylpyrimidine (**4a**) was also successfully synthesized starting from guanidine carbonate and ethyl-4,4,4-trifluoroacetoacetate.

The synthesis procedure to prepare the pyrimidine **4a** was modified since the trifluoromethyl group is an electron withdrawing group and increases the lipophilicity. The electron withdrawing effect of the trifluoromethyl group also facilitates the substitution of the 4-chloropyrimidine intermediate with ammonia under relatively mild conditions. The product **4a** was obtained as a pale-yellow solid in 82% overall yield (Figure 3.6).

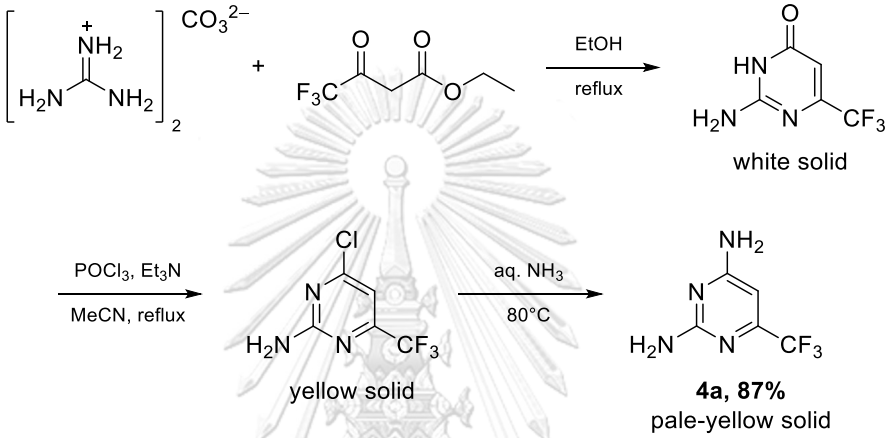
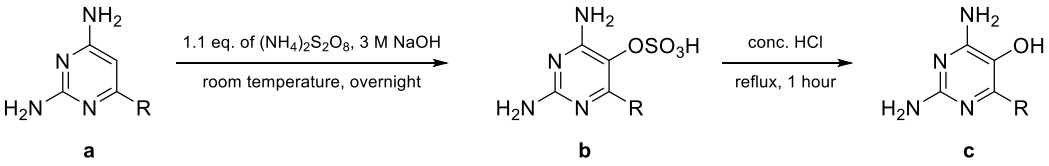


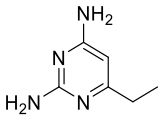
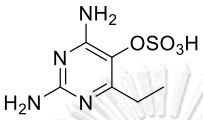
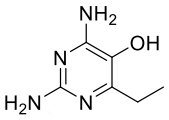
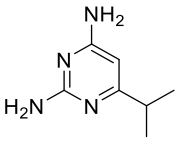
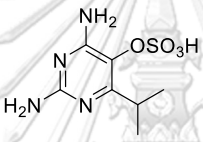
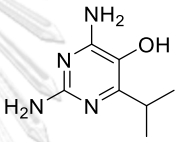
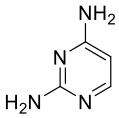
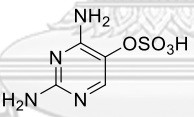
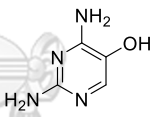
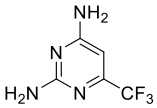
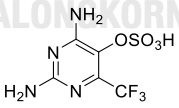
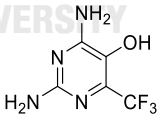
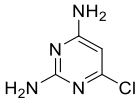
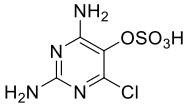
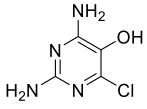
Figure 3.6 Synthetic route of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**).

The 2,4-diaminopyrimines (**2a-5a**) were oxidized in order to afford the corresponding hydroxypyrimidine products using the same conditions that was optimized for the oxidation of 2,4-diamino-6-ethylpyrimidine (**1a**) as summarized in Table 3.3. 2,4-Diamino-5-hydroxy-6-isopropylpyrimidine (**2c**) was successfully obtained in 58% overall yield as a brown solid. The yield of 2,4-diamino-5-hydroxypyrimidine (**3c**) was partially lost due to its solubility in water and was obtained in 43% overall yield. Even though two hydrogen atoms that could potentially be oxidized (C-5 and C-6) are present on 2,4-diaminopyrimidine (3a), the 5-hydroxypyrimidine **3c** was regioselectively obtained in which only the C-5 position was oxidized. Unfortunately, the two electron-deficient 2,4-diaminopyrimidines included in this study namely 2,4-diamino-6-trifluoromethylpyrimidine (**4a**) and 2,4-diamino-6-chloropyrimidine (**5a**) could not react to give the desired oxidation product.

Table 3.3 Substrate scope for Boyland-Sims oxidation of 2,4-diaminopyrimidines.

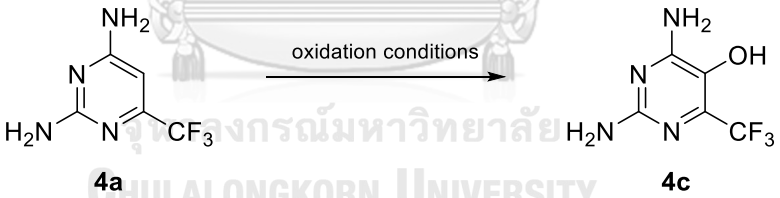


a **b** **c**

Substrate	Isolated Yields of compound b	Isolated Yields of compound c	Overall Isolated Yield
 **1a**	 **1b** 65%	 **1c** 81%	53%
 **2a**	 **2b** 67%	 **2c** 86%	58%
 **3a**	 **3b** 58%	 **3c** 75%	43%
 **4a**	 **4b** 0%	 **4c** 0%	no reaction
 **5a**	 **5b** 0%	 **5c** 0%	no reaction

3.2 Oxidation of 2,4-Diamino-6-trifluoromethylpyrimidine

Since electron-deficient 2,4-diaminopyrimidines including 2,4-diamino-6-trifluoromethylpyrimidine (**4a**) and 2,4-diamino-6-chloropyrimidine (**5a**) could not be oxidized by ammonium persulfate as oxidant to give the desired hydroxypyrimidines, oxidation of electron-deficient 2,4-diaminopyrimidines was further studied with other oxidants. 2,4-Diamino-6-trifluoromethylpyrimidine (**4a**) was chosen as a model compound since the trifluoromethyl group in this fluorinated pyrimidine facilitates reaction monitoring in a quantitative fashion using ^{19}F NMR technique. The reactions were performed at 0.05 mmol scale of the starting materials with various oxidants, additives, solvents, temperature and time with the aim to afford 2,4-diamino-5-hydroxy-6-trifluoromethylpyrimidine (**4c**) (Figure 3.7). The internal standard (trifluoroacetamide or trifluorotoluene) was added to the crude reaction product with the same equivalent to the starting material. Then the yields of the product were calculated by integrating the signal relative to the internal standard from ^{19}F NMR spectra.



4a **4c**

Figure 3.7 Oxidation of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**) to afford 2,4-diamino-5-hydroxy-6-trifluoromethylpyrimidine (**4c**).

Initially, 2,4-diamino-6-trifluoromethylpyrimidine (**4a**) was allowed to react under the previously reported direct C–H oxidation of aromatic compounds using phthaloyl peroxide.[39] Phthaloyl peroxide was simply prepared in one step starting from phthaloyl dichloride and sodium percarbonate. The fluorinated pyrimidine substrate was expected to react with phthaloyl peroxide to give the corresponding

phthaloyl ester. The ester was subsequently hydrolyzed to afford the hydroxypyrimidine **4c** (Figure 3.8).

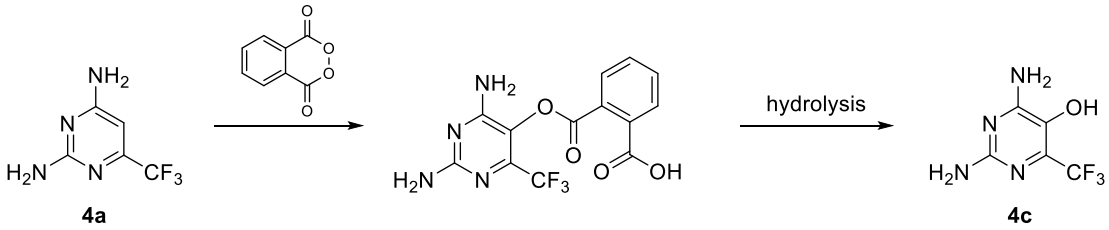


Figure 3.8 The proposed oxidation of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**) using phthaloyl peroxide.[39]

The ^{19}F NMR spectrum of the crude reaction product and the internal standard, trifluoroacetamide, showed three major signals (Figure 3.9). The singlet signals at –75.4 and –70.0 ppm correspond to the internal standard and the starting pyrimidine **4a**, respectively. An unknown ^{19}F NMR signal was also observed at –68.7 ppm. The reaction was reproduced at a larger scale of starting material (1.4 mmol) in order to characterize the product that gave the signal at –68.7 ppm. Instead of the expected hydroxylation product **4c**, a pale-yellow solid which was subsequently identified as 2,4-diamino-6-trifluoromethylpyrimidine-3-*N*-oxide (**4d**) by NMR and mass spectroscopy was isolated in 0.124 g from 1.4 mmol of the starting pyrimidine. It should be noted that the 2,4-diaminopyrimidine-3-*N*-oxides were previously obtained from the oxidation of 2,4-diaminopyrimidine with organic peroxide.[47-48]

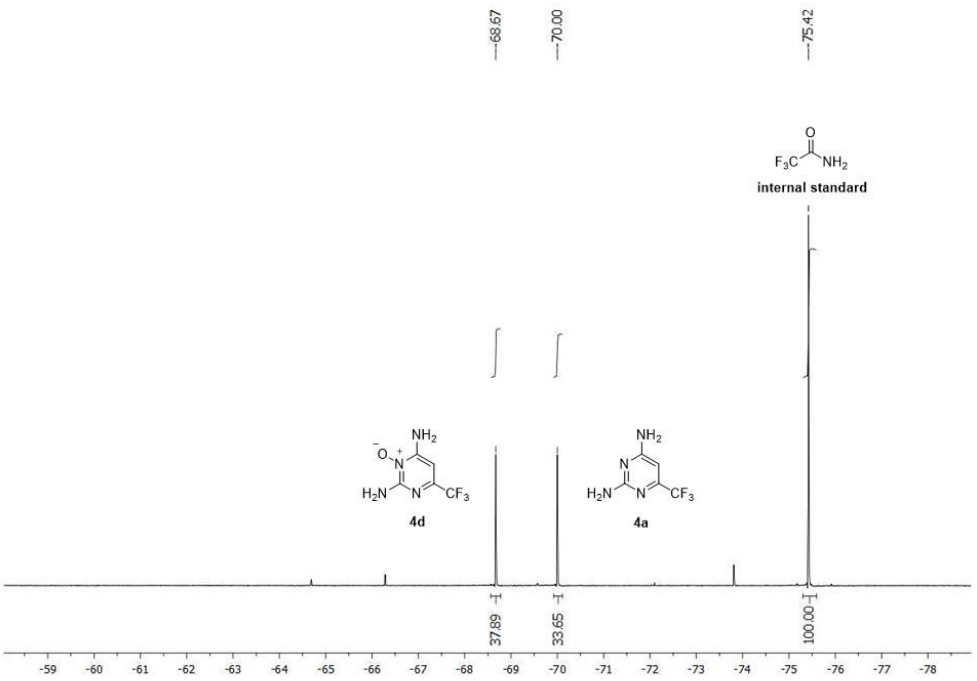


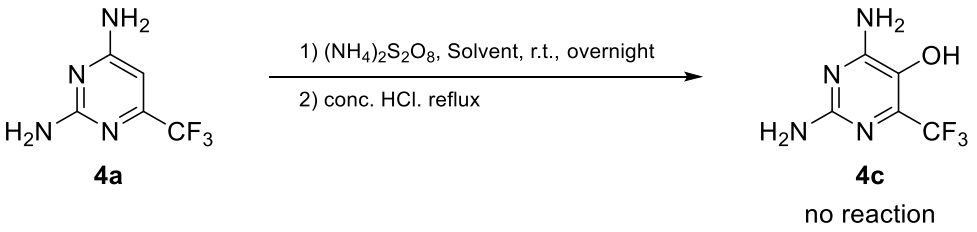
Figure 3.9 ^{19}F NMR spectrum of the crude reaction mixture and trifluoroacetamide as an internal standard.

3.2.1 Boyland-Sims Oxidation of 2,4-Diamino-6-trifluoromethylpyrimidine (4a)

Due to the failure of the Boyland-Sims oxidation of **4a** under the optimized conditions obtained from 2,4-diamino-6-ethylpyrimidine (**1a**), the reaction was re-optimized using **4a** as substrate at 0.05 mmol scale using ammonium persulfate in aqueous sodium hydroxide as oxidant. The substrate was allowed to react with the oxidant under various conditions as summarized in Table 3.4. The reaction under the previously reported conditions[35] using 1.5 equivalents of ammonium persulfate in 150 µL of 3 M sodium hydroxide solution led to complete consumption of the starting material (entry 1). However, a new signal of an unidentified species at –73.5 ppm was observed without any signals of the hydroxypyrimidine (**4c**) and the *N*-oxide (**4d**). The signal of the desired product **4c** was still not observed when the concentration of the substrate was reduced (entry 2) or the concentration of sodium hydroxide was increased (entry 3). Replacing sodium hydroxide with potassium hydroxide also did not yield the hydroxypyrimidine (entry 4). As the trifluoromethyl group increased the lipophilicity of the pyrimidine substrate, organic solvents such as acetonitrile, acetone, dimethylformamide, and dimethyl sulfoxide were employed as co-solvents to improve the solubility of the starting material. However, none of these conditions yielded the desired product. It should be noted that the addition of organic solvents might decrease the solubility of ammonium persulfate and led to the lower conversion of the starting material (entries 5-8).

The reaction was also performed in a weaker base such as a 30% wt/vol aqueous ammonia solution, but the result was similar (entry 9). The reaction performed under an inert atmosphere with the aim to avoid suppression of the radical pathway by molecular oxygen also did not yield the desired product (entry 10). Variation of the amount of ammonium persulfate also gave no expected product but caused decomposition of the starting material. The lower equivalents of oxidant (1.2 equivalents) decreased the decomposition, while higher equivalents of oxidant (2.0 equivalents) led to complete consumption of the starting material (entries 12-13).

Table 3.4 Oxidation of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**) with ammonium persulfate



4a **4c** no reaction

Entry	Solvent	Conversion (%)[c]	Yield (%)[c]
1	3 M NaOH	96	0
2[a]	3 M NaOH	100	0
3	6 M NaOH	74	0
4	3 M KOH	100	0
5	6 M NaOH:MeCN = 1:1	47	0
6	6 M NaOH:Acetone = 1:1	34	0
7	6 M NaOH:DMF = 1:1	26	0
8	6 M NaOH:DMSO = 1:1	96	0
9	NH_3 in H_2O (30% by mass)	100	0
10[b]	3 M NaOH	98	0
11	3 M NaOH (1.2 equiv. of $(NH_4)_2S_2O_8$)	87	0
12	3 M NaOH (2.0 equiv. of $(NH_4)_2S_2O_8$)	100	0

[a]300 µL of solvent, [b]reaction under N_2 atmosphere, [c]Yields and conversions were determined by ^{19}F NMR spectroscopy with trifluoroacetamide as internal standard.

The Boyland-Sims oxidation of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**) was reproduced in larger scale of the starting material with the aim to isolate and identify the product. The reaction was performed at 1.00 mmol scale of the starting material using the conditions in Table 3.4 entry 1. The pyrimidinyl hydrogen sulfate product was not obtained after the pyrimidine substrate was treated with

ammonium persulfate in aqueous basic solution. The reaction mixture was then concentrated and allowed to reflux with concentrated hydrochloric acid. The residual crude reaction product after solvent removal was dissolved in deuterium oxide (D_2O) and characterized by NMR spectroscopy. The signal of an unidentified product was observed at −75.4 ppm in ^{19}F NMR. This is the same signal was previously observed at −73.5 ppm when the ^{19}F NMR spectra was recorded in DMSO-d_6. The unidentified product gave two singlet signals in ^{1}H NMR and four signals in ^{13}C NMR suggesting that the pyrimidine substrate might be decomposed to a four-carbon compound (Figure 3.10).

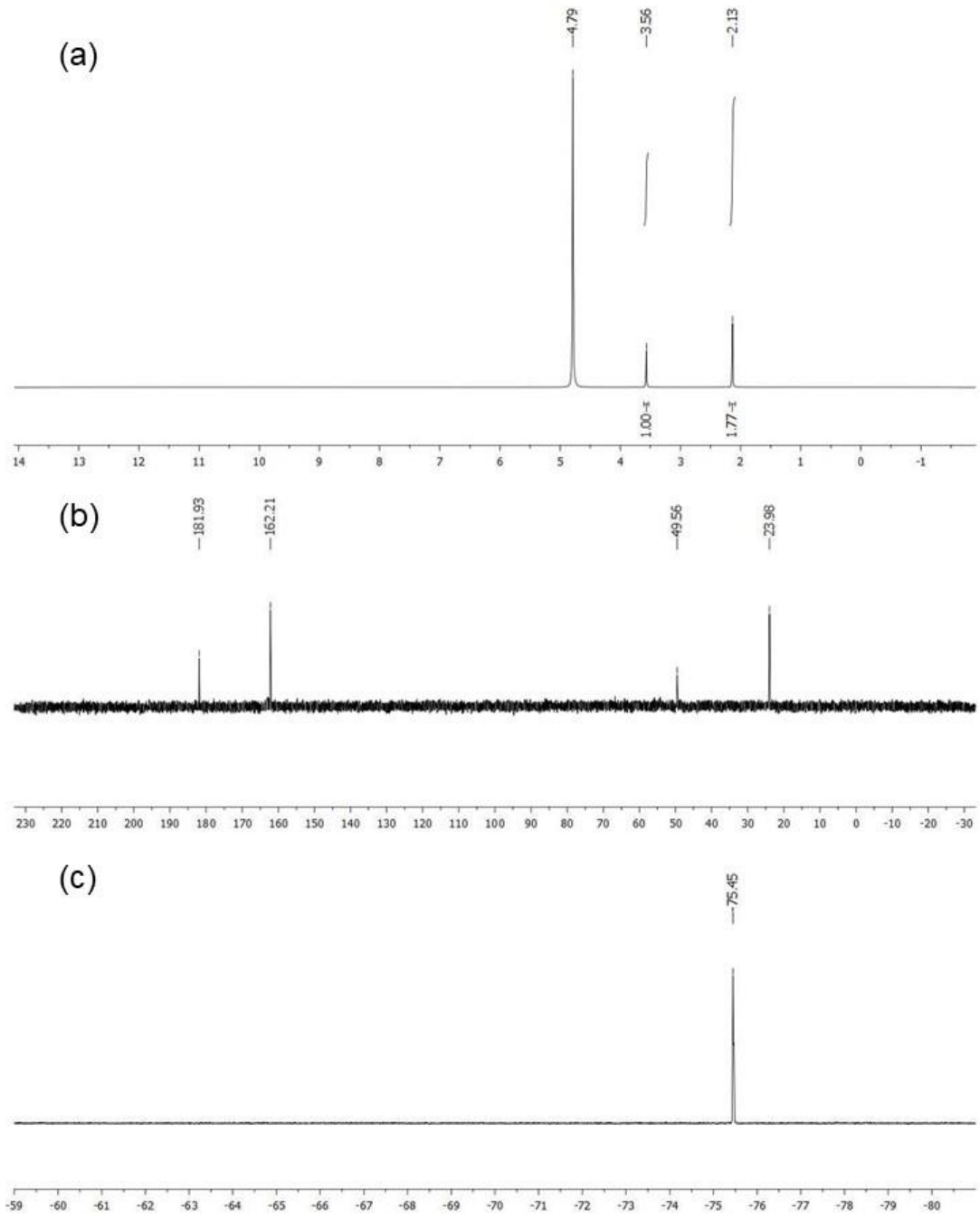
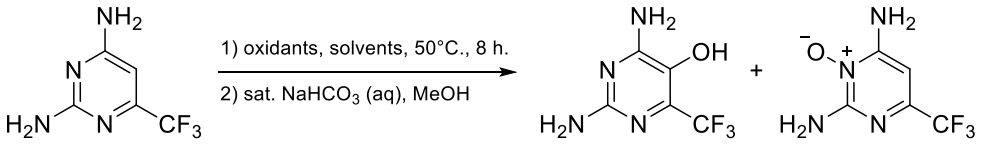


Figure 3.10 NMR spectra of crude product from the oxidation of 2,4-diamino-6-trifluoromethylpyrimidine with ammonium persulfate. (a) ^{1}H NMR (b) ^{13}C NMR and (c) ^{19}F NMR spectra.

3.2.2 Oxidation of 2,4-Diamino-6-trifluoromethylpyrimidine (4a) with Various Oxidants

Since the Boyland-Sims oxidation did not successfully yield the expected hydroxypyrimidine product **4c**, various other oxidants were screened as summarized in Table 3.5. Firstly, the reported direct C–H oxidation conditions[39] using phthaloyl peroxide as oxidant was applied. The fluorinated substrate was oxidized by phthaloyl peroxide in trifluoroethanol (TFE) to give the *N*-oxide **4d** in 38% without showing any sign of the desired product **4c** was not observed (entry 1). Similar results were obtained when benzoyl peroxide and *p*-nitrobenzoyl peroxide were employed as oxidants in acetic acid. The major product in both cases was the *N*-oxide which was obtained in 13% and 68%, respectively (entries 2-3). *p*-Nitrobenzoyl peroxide bearing the electron-withdrawal nitro group gave high yield of the *N*-oxide. It also showed the highest reactivity comparing to the other peroxides. Oxidation with other organic oxidants such as diacetoxyiodobenzene (PIDA) and [bis(trifluoroacetoxy)iodo]benzene (PIFA) were also attempted. Although the fluorinated pyrimidine substrate was partially consumed, the expected hydroxypyrimidine and the *N*-oxide were not obtained (entries 4-5).

Table 3.5 Oxidation of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**) with various oxidants



4a **4c** (not obtained) **4d**

Entry	Oxidant	Solvent	Conversion (%)[a]	Yield (%)[a]	
				4c	4d
1	Phthaloyl peroxide	TFE	66	0	38
2	Benzoyl peroxide	HOAc	15	0	13
3	*p*-Nitrobenzoyl peroxide	HOAc	68	0	68
4	PIDA	HOAc	29	0	0
5	PIFA	HOAc	74	0	0
6[b,c]	NaOAc (3.4 equiv.), Br_2 (1.0 equiv.)	DCM	100	0	8
7[b,d]	NH_4OAc (3.4 equiv.), Br_2 (1.0 equiv.)	MeCN	78	0	0

[a]Yields and conversions were determined by ^{19}F NMR spectroscopy with trifluoroacetamide as internal standard. [b]Reaction was set up at 0°C then left at room temperature. [c]20% yield of 2,4-diamino-5-bromo-6-trifluoromethylpyrimidine (**4f**). [d]44% yield of 2,4-diamino-5-bromo-6-trifluoromethylpyrimidine (**4f**)

The reaction was also performed under the reported conditions using sodium acetate and liquid bromine.[37] The substrate was completely consumed resulting 8% yield of the *N*-oxide (entry 6) and 20% of an unknown product which gave the ^{19}F NMR signal at –66.1 ppm. The signal of the unknown product was increased to 44% when replacing sodium acetate in dichloromethane with ammonium acetate in acetonitrile, but the starting material was not completely consumed (entry 7). The reaction was repeated in a larger scale and the unknown product was isolated as a pale-yellow solid. It was fully characterized and identified as 2,4-diamino-5-bromo-6-

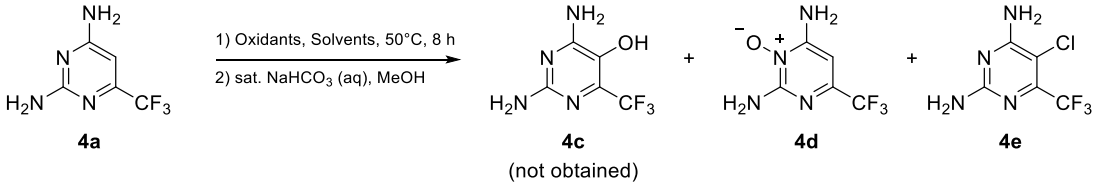
trifluoromethylpyrimidine (**4f**) (Figure 3.11). It should be noted that bromination at C-5 position was more favored since the pyrimidine ring was deactivated by electron-withdrawal trifluoromethyl group. In addition to this, the brominated pyrimidine was reported to be a product under these conditions in literature when 6-chloropyrimidine was used as a starting material.[37]

4a **4d** **4f**

Figure 3.11 Reaction of 2,4-diamino-6-trifluoromethylpyrimidine with sodium acetate and liquid bromine.

The reaction of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**) with organic peroxides including: phthaloyl peroxide, benzoyl peroxide, and *p*-nitrobenzoyl peroxide were further attempted with the aim to afford 2,4-diamino-5-hydroxy-6-trifluoropyrimidine (**4c**). Firstly, the reactions were performed using organic peroxides in various solvents as summarized in Table 3.6. The reactions were performed at 0.05 mmol scale of the starting material **4a** using 1.5 equivalents of organic peroxides including phthaloyl peroxide, benzoyl peroxide and *p*-nitrobenzoyl peroxide as oxidants.

Table 3.6 Oxidation of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**) with organic peroxides.



Entry	Oxidant	Solvent	Conversion (%)[a]	Yield (%)[a]		
				4c	4d	4e[e]
1	Phthaloyl peroxide	TFE	66	0	38	2
2	Phthaloyl peroxide	HFIP	57	0	16	6
3	*p*-Nitrobenzoyl peroxide	TFE	71	0	19	0
4	Benzoyl peroxide	HOAc	15	0	13	0
5	*p*-Nitrobenzoyl peroxide	HOAc	68	0	68	0
6[b]	*p*-Nitrobenzoyl peroxide	HOAc	100	0	29	0
7	Phthaloyl peroxide	HOAc	63	0	40	21
8[b]	Phthaloyl peroxide	HOAc	99	0	2	22
9[c]	Phthaloyl peroxide	HOAc	95	0	18	15
10[d]	Phthaloyl peroxide	HOAc	74	0	47	4
11	Phthaloyl peroxide	PPA	70	0	25	1
12	Phthaloyl peroxide	DCM	71	0	0	0
13	Phthaloyl peroxide	DCE	66	0	0	0
14	Phthaloyl peroxide	Toluene	68	0	15	0
15	Phthaloyl peroxide	THF	55	0	0	1
16	Phthaloyl peroxide	*i*PrOH	49	0	2	5
17	Phthaloyl peroxide	DMF	88	0	0	4
18	Phthaloyl peroxide	DMSO	38	0	0	0

[a]Yields and conversions were determined by ^{19}F NMR spectroscopy with trifluoroacetamide as internal standard. [b]3.0 equivalents of oxidants. [c]Reaction at room temperature overnight. [d]Acetic acid was further dried with molecular sieve. [e]Chlorination by the residual chloride ion in phthaloyl peroxide.

The starting pyrimidine **4a** was oxidized by phthaloyl peroxide and gave the *N*-oxide **4d** in 38% and 16% yield in both fluorinated solvents employed, trifluoroethanol (TFE) and hexafluoroisopropanol (HFIP), respectively (entries 1-2). The signal of the *N*-oxide **4d** was observed in ^{19}F NMR spectra along with the major signal of an unknown product at –66.2 ppm. The yield of the unknown product increased to 21% when the reaction was performed in acetic acid (entry 7). The reaction was repeated in larger scale using the conditions shown in Table 3.6 entry 7 (1.5 equivalents of phthaloyl peroxide in acetic acid). The pale-yellow solid of another product was isolated in 18% and 16% in 2.0 and 5.0 mmol scales of the starting trifluoromethylpyrimidine, respectively. It was fully characterized by NMR and mass spectrometry and was identified as 2,4-diamino-5-chloro-6-trifluoromethylpyrimidine (**4e**). With benzoyl peroxide and *p*-nitrobenzoyl peroxide as oxidants in trifluoroethanol or acetic acid gave only the *N*-oxide as a product (entries 3-6). No chlorinated pyrimidine **4e** was observed. This led us to propose that the chlorine atom might originate from the residual chloride ion from the synthesis of phthaloyl peroxide from phthaloyl dichloride.

The oxidation of the 2,4-diamino-6-trifluoromethylpyrimidine substrate **4a** was further performed under various conditions. Increasing the equivalents of phthaloyl peroxide led to complete disappearance of the starting material, but the chlorinated pyrimidine **4e** was still observed as a major product (entry 8). The reaction in acetic acid at room temperature overnight gave lower yields of both the *N*-oxide and the chlorinated pyrimidine, although the starting material was almost completely consumed. (entry 9). The reaction was also performed in acetic acid which was further dried with molecular sieve (entry 10). In this case, the yield of the chloropyrimidine was reduced, but the *N*-oxide was observed as a major product. Replacing acetic acid with propionic acid (PPA) resulted in a lower yield of the *N*-oxide (entry 11). It should be noted that using polar solvents might increase the solubility of the residual chloride ion the system and led to chlorination of the pyrimidine substrate.

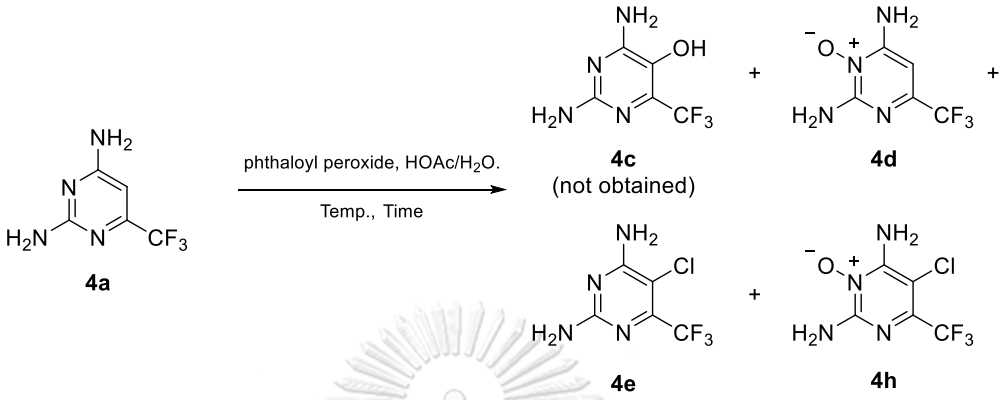
Other organic solvents including dichloromethane (DCM), dichloroethane (DCE), toluene, tetrahydrofuran (THF), isopropanol (*i*PrOH), dimethylformamide (DMF),

and dimethyl sulfoxide (DMSO) were also tested with the phthaloyl peroxide oxidation (entries 12-18). The *N*-oxide was obtained in 15% and 2% yield when using toluene and isopropanol as a solvent, respectively. The chlorinated pyrimidine was obtained in less than 5% yield since the residual chloride ion had possessed low solubility in these organic solvents. However, the desired hydroxypyrimidine **4c** could still not be obtained.

3.2.2.1 Addition of Water

The reactions were also performed in dilute acetic acid using phthaloyl peroxide as the oxidant by adding water to the reaction mixture as summarized in Table 3.7. The quantities of water did not affect the yield of products. The reactions with 1.5 equivalents of phthaloyl peroxide did not yield the hydroxypyrimidine **4c** but gave the *N*-oxide **4d** in 57-65%. However, a new ^{19}F NMR signal was observed at –65.3 ppm in 15-18% (entries 1-5). Increasing the equivalents of phthaloyl peroxide affected the yield of a new product. However, the yield was decreased when the reactions were performed at higher temperature or left overnight (entries 6-9).

Table 3.7 Oxidation of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**) with phthaloyl peroxide in dilute acetic acid.



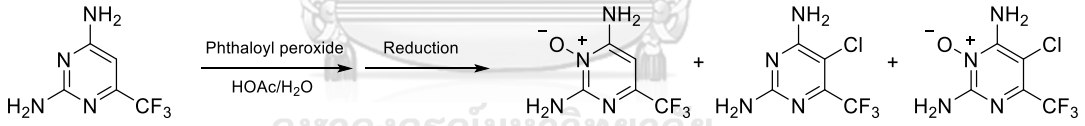
Entry	HOAc:H_2O	Time (h.)	Temp. (°C)	Conversion (%)[a]	Yield (%)[a]			
					4c	4d	4e[c]	4h[c]
1	4:1	8	r.t.	95	0	64	5	17
2	3:1	8	r.t.	93	0	63	6	16
3	2:1	8	r.t.	93	0	57	5	18
4	1:1	8	r.t.	83	0	65	5	15
5	2:1	overnight	r.t.	88	0	58	3	17
6[b]	2:1	8	r.t	95	0	36	3	72
7[b]	2:1	8	50	100	0	12	3	46
8[b]	2:1	8	80	100	0	11	3	50
9[b]	2:1	overnight	r.t.	100	0	34	0	43

[a]Yields and conversions were determined by ^{19}F NMR spectroscopy with trifluorotoluene as internal standard. [b]3.0 equivalents of phthaloyl peroxide. [c]Chlorination by the residual chloride ion in phthaloyl peroxide.

Since the addition of water increased solubility of the residual chloride ion in phthaloyl peroxide and also increased the reactivity of the oxidant, the signal of chloropyrimidine *N*-oxide **4h** was observed as the major product at –65.3 ppm in ^{19}F NMR spectra. The chloropyrimidine *N*-oxide **4h** was not isolated but it was directly reduced to the chloropyrimidine **4e** using the previously reported reduction

conditions for *N*-oxides.[49-50] The chloropyrimidine *N*-oxide **4h** was generated *in situ* from 0.05 mmol of the starting pyrimidine **4a** using the conditions shown in Table 3.7 entry 6 and reduced by zinc dust/ammonium formate or phenylboronic acid as shown in Table 3.8. The chloropyrimidine **4e** was obtained in higher yield after the reaction crude from the oxidation with phthaloyl peroxide was reduced. Using of phenylboronic acid as a reductant gave the higher yield of the chloropyrimidine **4e**. Increasing equivalent of phenylboronic acid could yield the chlorinated pyrimidine **4e** in 34% with the complete consumption of the starting pyrimidine (entry 2). However, some starting material **4a** was recovered when using 3.0 equivalents of phenylboronic acid or zinc dust/ammonium formate as reductants (entries 1 and 3). It could be due to the reduction of *N*-oxide **4d** to the starting pyrimidine **4a** since the pyrimidine substrate was consumed in 95% by the oxidation with phthaloyl peroxide (Table 3.7, entry 6).

Table 3.8 Reduction of the chlorinated 2,4-diamino-6-trifluoromethylpyrimidine-3-*N*-oxide.



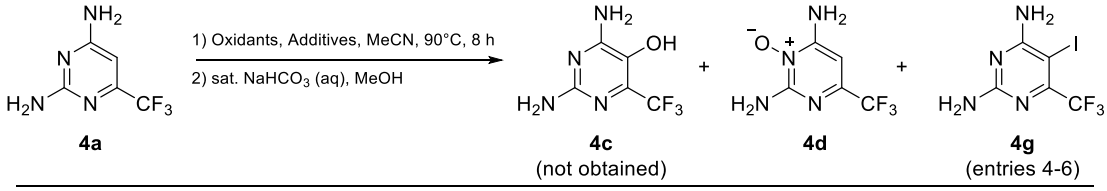
Entry	Reductants	Equiv. of Reductant	Conversion (%)[a,b]	Yield (%)[a] 4d	4e[c]	4h[c]
1	Phenylboronic acid	3.0	92	10	25	12
2	Phenylboronic acid	5.0	100	0	34	12
3	Zn dust $HCOONH_4$	2.0 3.0	84	3	15	34

[a]Yields and conversions were determined by ^{19}F NMR spectroscopy with trifluorotoluene as internal standard. [b]Conversions based on 2,4-diamino-6-trifluoromethylpyrimidine (**4a**). [c] Chlorination by the residual chloride ion in phthaloyl peroxide.

3.2.2.2 Addition of Radical Initiators

Radical initiators including molecular iodine (0.3 equiv.) and azobisisobutyronitrile (AIBN) (0.1 equiv.) were added to the reaction with the aim to promote the formation of free radicals from the organic peroxides and might force the reaction to proceed through a radical mechanism. The reactions were heated in 150 µL of acetonitrile using 1.5 equivalents of organic peroxides and radical initiators as additives as shown in Table 3.9. The reaction without any radical initiators did not yield the product and the starting pyrimidine was partially decomposed (entries 1-3). The reaction under the reported conditions[38] in the presence of iodine did not yield the hydroxypyrimidine **4c** or *N*-oxide **4d**. However, the ^{19}F NMR signal of the product was observed at –65.8 ppm (entries 4-6). This product was later isolated from the reaction in larger scale using *p*-nitrobenzoyl peroxide as oxidant and was confirmed to be 2,4-diamino-5-iodo-6-trifluoromethylpydimidine (**4g**) by NMR and mass spectroscopy. AIBN was also used as a radical initiator. The expected hydroxypyrimidine product **4c** was still not obtained, but the *N*-oxides was obtained in 4% when using *p*-nitrobenzoyl peroxide as oxidant (entries 7-9). Additionally, the reactions were allowed to proceed under irradiation of incandescent light (30 W, 2080 lm) in order to generate radicals from the organic peroxides. Although the substrate was partially consumed, only the *N*-oxide **4d** was observed when using benzoyl peroxide or *p*-nitrobenzoyl peroxide as oxidants (entries 10-12).

Table 3.9 Oxidation of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**) with organic peroxides in acetonitrile.



Entry	Oxidants	Additive	Conversion (%)[a]	Yield (%)[a]		
				4c	4d	4g
1	Phthaloyl peroxide	-	62	0	0	0
2	Benzoyl peroxide	-	36	0	0	0
3	*p*-Nitrobenzoyl peroxide	-	40	0	0	0
4	Phthaloyl peroxide	I_2	87	0	0	36
5	Benzoyl peroxide	I_2	38	0	0	28
6	*p*-Nitrobenzoyl peroxide	I_2	98	0	0	73
7	Phthaloyl peroxide	AIBN	63	0	0	0
8	Benzoyl peroxide	AIBN	35	0	0	0
9	*p*-Nitrobenzoyl peroxide	AIBN	82	0	4	0
10[b]	Phthaloyl peroxide	-	54	0	0	0
11[b]	Benzoyl peroxide	-	52	0	17	0
12[b]	*p*-Nitrobenzoyl peroxide	-	62	0	16	0

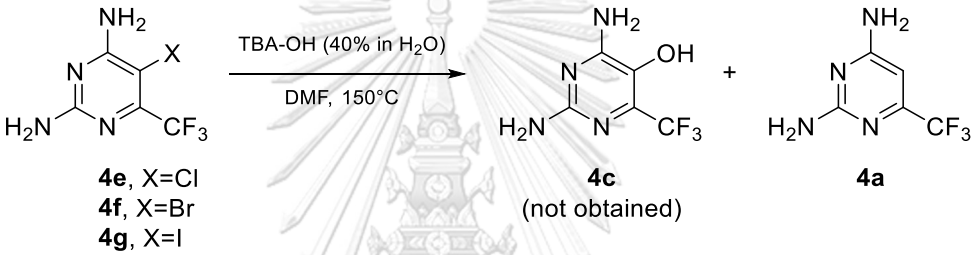
[a]Yields and conversions were determined by ^{19}F NMR spectroscopy with trifluoroacetamide as internal standard. [b]Reactions under incandescent light.

3.2.3 Attempt to Convert the Halogenated Trifluoromethylpyrimidines to the Desired Product

As 2,4-diamino-5-chloro-6-trifluoromethylpydimidine (**4e**), 2,4-diamino-5-bromo-6-trifluoromethylpydimidine (**4f**), and 2,4-diamino-5-iodo-6-trifluoromethylpydimidine (**4g**) were obtained from the reactions, these side products were treated with aqueous solution of tetrabutylammonium hydroxide (TBA-OH) with the aim to convert the halogen to the hydroxy group through nucleophilic aromatic

substitution. Unfortunately, the iodinated trifluoromethylpyrimidine (**4g**) was almost completely converted back to the starting 2,4-diamino-6-trifluoromethylpyrimidine (**4a**). Similar reaction employing from the brominated trifluoromethylpyrimidine (**4f**) as the substrate also gave the starting pyrimidine **4a** in 49% yield, while no reaction was observed when the chlorinated trifluoromethylpyrimidine (**4e**) was employed as the starting material (Table 3.10).

Table 3.10 Reaction of halogenated 2,4-diamino-6-trifluoromethylpydimidine with tetrabutylammonium hydroxide.



4e, X=Cl
4f, X=Br
4g, X=I

4c (not obtained)

4a

Entry	Substrate	Conversion (%)[a]	Yield (%)[a] 4c	4a
1	**4e**	14	0	0
2	**4f**	50	0	49
3	**4g**	98	0	97

[a]Yields and conversions were determined by ^{19}F NMR spectroscopy with trifluorotoluene as internal standard.

The reaction probably occurred through the attack of hydroxide ion to the halogen atom on the pyrimidine ring to generate the electron-deficient pyrimidine anion as the leaving group. Then, the anion abstracted a proton from water to afford 2,4-diamino-6-trifluoromethylpyrimidine (**4a**) as the product (Figure 3.12). Noteworthily, the iodinated substrate gave the highest yield of the product since C–I (209 kJ/mol) bond is weaker than C–Br (280 kJ/mol) and C–Cl (397 kJ/mol) bonds.[51]

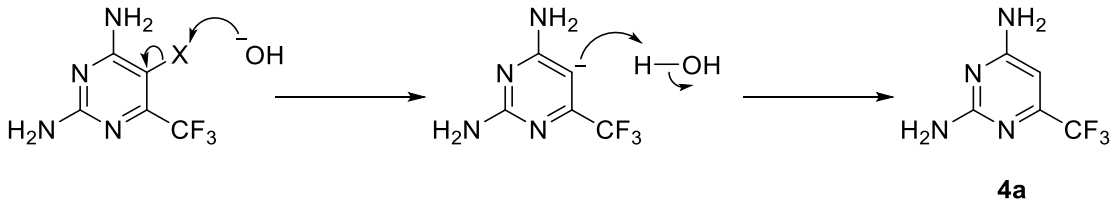


Figure 3.12 Plausible mechanisms of the reaction of halogenated 2,4-diamino-6-trifluoromethylpydimidine with tetrabutylammonium hydroxide.

3.3 Summary

2,4-Diaminopyrimidines could be oxidized to 2,4-diamino-5-hydroxypyrimidines through Boyland-Sims oxidation using ammonium persulfate in aqueous basic solvent. However, the persulfate oxidation could only be applied with 2,4-diaminopyrimidines without substituent or with electron-donating alkyl group at the C-6 position. The electron-deficient 2,4-diaminopyrimidines bearing chloro or trifluoromethyl group at the position C-6 could not undergo the Boyland-Sims oxidation.

Oxidation of the trifluoromethylated 2,4-diaminopyrimidine is highly desirable since the trifluoromethyl group has been frequently employed in drug discovery due to its ability to improve bioavailability of the drug.[52] The success of the improved oxidation would be useful for development of novel 2,4-diaminopyrimidine antifolates with better activities and bioavailability. Unfortunately, when the oxidation of 2,4-diamino-6-trifluoromethylpyrimidine as a representative electron-deficient pyrimidine was attempted with various oxidants, the expected 2,4-diamino-5-hydroxy-6-trifluoromethylpyrimidine was not obtained. It still remains challenging to find out the method to oxidize such the electron-deficient pyrimidines.

CHAPTER IV
CONCLUSION

2,4-Diamino-5-hydroxypyrimidines could be obtained from Boyland-Sims oxidation of 2,4-diaminopyrimidine. The pyrimidine substrate was firstly converted to a pyrimidinyl hydrogen sulfate intermediate using ammonium persulfate in aqueous basic media followed by hydrolysis to afford the hydroxypyrimidine. This procedure suffers low yield and poor reproducibility. In this work, the conditions for Boyland-Sims oxidation of 2,4-diaminopyrimidines were optimized using 2,4-diamino-6-ethylpyrimidine as a model compound. The parameters that could affect the yield of the hydroxylated product were also investigated. The yield of the product as calculated from ^{1}H NMR with internal standard decreased with increasing equivalents of ammonium persulfate. The concentration of substrate did not affect the yield significantly. The solubility of the pyrimidine substrate decreased with increasing concentration of the base and this led to incomplete reaction. For the base, sodium hydroxide gave better yields than potassium hydroxide and lithium hydroxide, respectively. The Boyland-Sims oxidation of 2,4-diamino-6-ethylpyrimidine was reproduced at 3 mmol scale using the optimized conditions (1.1 equivalents of ammonium per sulfate in 3 M sodium hydroxide solution) and gave the desired hydroxylated product in 53% overall yield. The same conditions were also applied to other 2,4-diaminopyrimidine derivatives bearing different substituents at C-6 position. The hydroxypyrimidine was obtained in satisfactory yields from 2,4-diaminopyrimidine without substituent or with isopropyl group at the C-6 position. Unfortunately, the electron-deficient 2,4-diaminopyrimidines bearing chloro or trifluoromethyl group at the C-6 position could not undergo the Boyland-Sims oxidation.

Oxidation of the electron-deficient 2,4-diaminopyrimidines was further studied using 2,4-diamino-6-trifluoromethylpyrimidine as a model compound. Oxidation of the trifluoromethylated pyrimidine substrate was attempted with various oxidants under different conditions. The Boyland-Sims oxidation of the fluorinated pyrimidine

was re-optimized and did not yield the expected hydroxylated product. Oxidation with organic peroxides such as phthaloyl peroxide, benzoyl peroxide, and *p*-nitrobenzoyl peroxide did not yield the expected hydroxypyrimidine but gave 2,4-diamino-6-trifluoromethylpyrimidine-3-*N*-oxide as a major product. 2,4-Diamino-5-chloro-6-trifluoromethylpyrimdine was observed when using phthaloyl peroxide as the oxidant. The chlorine atom might originate from the residual chloride ion from the synthesis of phthaloyl peroxide from phthaloyl dichloride. The 5-chlorinated pyrimidine was obtained in higher yield when using polar solvents such as acetic acid. The *N*-oxide of the chloropyrimidine was observed when using phthaloyl peroxide in aqueous solution of acetic acid and this could be reduced back to the chloropyrimidine by heating with phenylboronic acid. Addition of radical initiators such as AIBN or molecular iodine with the aim to induce the reaction to proceed through a radical mechanism did not gave the expected hydroxypyrimidine. However, the 5-brominated and 5-iodinated pyrimidines were obtained from the reaction involving liquid bromine and molecular iodine, respectively. Conversion of the C5-halogenated pyrimidines to the hydroxypyrimidine was also attempted, but the starting 2,4-diamino-6-trifluoromethylpyrimidine was obtained instead.

The scope of oxidation of 2,4-diaminopyrimidines to 2,4-diamino-5-hydroxypyrimidines is therefore still limited to electron rich 2,4-diaminopyrimidines. Oxidation of electron-deficient 2,4-diaminopyrimidines still remains challenging, especially the trifluoromethylated 2,4-diaminopyrimidine which is highly desirable since the trifluoromethyl group has been frequently employed in drug discovery due to its ability to improve bioavailability of the drug. Thus, the success of this improved oxidation in the future would be useful for development of novel 2,4-diaminopyrimidine antifolates with better activities and bioavailability.

REFERENCES

1. Dox, A. W. Synthetic Hypnotics of the Barbituric Acid Series. *J. Am. Pharm. Assoc.* **1923,** *12,* 602.
2. Wiley, P. F.; MacKellar, F. A. Sparsomycin, Structure and Chemistry. *J. Org. Chem.* **1976,** *41,* 1858-1862.
3. Reddick, J. J.; Saha, S.; Lee, J.; Melnick, J. S.; Perkins, J.; Begley, T. P. The Mechanism of Action of Bacimethrin, a Naturally Occurring Thiamin Antimetabolite. *Bioorg. Med. Chem. Lett.* **2001,** *11,* 2245-2248.
4. Sharma, P.; Rane, N.; Gurram, V. K. Synthesis and QSAR Studies of Pyrimido[4,5-D]pyrimidine-2,5-dione Derivatives as Potential Antimicrobial Agents. *Bioorg. Med. Chem. Lett.* **2004,** *14,* 4185-4190.
5. Prakash, O.; Bhardwaj, V.; Kumar, R.; Tyagi, P.; Aneja, K. R. Organoiodine (III) Mediated Synthesis of 3-Aryl/Hetryl-5,7-dimethyl-1,2,4-triazolo[4,3-a]pyrimidines as Antibacterial Agents. *Eur. J. Med. Chem.* **2004,** *39,* 1073-1077.
6. Agarwal, N.; Srivastava, P.; Raghuwanshi, S. K.; Upadhyay, D. N.; Sinha, S.; Shukla, P. K.; Ji Ram, V. Chloropyrimidines as a New Class of Antimicrobial Agents. *Biorg. Med. Chem.* **2002,** *10,* 869-874.
7. Juby, P. F.; Hudyma, T. W.; Brown, M.; Essery, J. M.; Partyka, R. A. Antiallergy Agents. 1. 1,6-Dihydro-6-oxo-2-phenylpyrimidine-5-carboxylic Acids and Esters. *J. Med. Chem.* **1979,** *22,* 263-269.
8. Xie, F.; Zhao, H.; Zhao, L.; Lou, L.; Hu, Y. Synthesis and Biological Evaluation of Novel 2,4,5-Substituted Pyrimidine Derivatives for Anticancer Activity. *Bioorg. Med. Chem. Lett.* **2009,** *19,* 275-278.
9. Kaldrikyan, M. A.; Grigoryan, L. A.; Geboyan, V. A.; Arsenyan, F. G.; Stepanyan, G. M.; Garibdzhanyan, B. T. Synthesis and Antitumor Activity of Some Disubstituted 5-(3-Methyl-4-alkoxybenzyl)pyrimidines. *Pharm. Chem. J.* **2000,** *34,* 521-524.
10. Agarwal, N.; Raghuwanshi, S. K.; Upadhyay, D. N.; Shukla, P. K.; Ram, V. J. Suitably Functionalised Pyrimidines as Potential Antimycotic Agents. *Bioorg. Med. Chem. Lett.* **2000,** *10,* 703-706.

11. Keri, R. S.; Hosamani, K. M.; Shingalapur, R. V.; Hugar, M. H. Analgesic, Anti-Pyretic and DNA Cleavage Studies of Novel Pyrimidine Derivatives of Coumarin Moiety. *Eur. J. Med. Chem.* **2010,** *45,* 2597-2605.

12. Antre, R. V.; Cendilkumar, A.; Goli, D.; Andhale, G. S.; Oswal, R. J. Microwave Assisted Synthesis of Novel Pyrazolone Derivatives Attached to A Pyrimidine Moiety and Evaluation of Their Anti-inflammatory, Analgesic and Antipyretic Activities. *Saudi Pharmaceutical Journal* **2011,** *19,* 233-243.

13. Sondhi, S. M.; Jain, S.; Dwivedi, A. D.; Shukla, R.; Raghubir, R. Synthesis of Condensed Pyrimidines and Their Evaluation for Anti-inflammatory and Analgesic Activities. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **2008,** *47B,* 136-143.

14. Amir, M.; Javed, S. A.; Kumar, H. Pyrimidine as Antiinflammatory Agent: A Review. *Indian J. Pharm. Sci.* **2007,** *69,* 337-343.

15. Siddiqui, A. B.; Trivedi, A. R.; Kataria, V. B.; Shah, V. H. 4,5-Dihydro-1H-pyrazolo[3,4-d]pyrimidine Containing Phenothiazines as Antitubercular Agents. *Bioorg. Med. Chem. Lett.* **2014,** *24,* 1493-1495.

16. Singh, K.; Kaur, T. Pyrimidine-based Antimalarials: Design Strategies and Antiplasmodial Effects. *MedChemComm.* **2016,** *7,* 749-768.

17. Kumar, B.; Kaur, B.; Kaur, J.; Parmar, A.; Anand, R. D.; Kumar, H. Thermal/Microwave Assisted Synthesis of Substituted Tetrahydropyrimidines as Potent Calcium Channel Blockers. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **2002,** *41B,* 1526-1530.

18. Rodrigues, A. L. S.; Rosa, J. M.; Gadotti, V. M.; Goulart, E. C.; Santos, M. M.; Silva, A. V.; Sehnem, B.; Rosa, L. S.; Gonçalves, R. M.; Corrêa, R.; Santos, A. R. S. Antidepressant-like and Antinociceptive-like Actions of 4-(4'-Chlorophenyl)-6-(4''-methylphenyl)-2-hydrazinepyrimidine Mannich Base in Mice. *Pharmacol. Biochem. Behav.* **2005,** *82,* 156-162.

19. Tani, J.; Yamada, Y.; Oine, T.; Ochiai, T.; Ishida, R.; Inoue, I. Studies on Biologically Active Halogenated Compounds. 1. Synthesis and Central Nervous System Depressant Activity of 2-(Fluoromethyl)-3-Aryl-4(3H)-quinazolinone Derivatives. *J. Med. Chem.* **1979,** *22,* 95-99.

20. Ashley, E. A.; Pyae Phyo, A.; Woodrow, C. J. Malaria. *The Lancet* **2018,** *391,* 1608-1621.

21. Ferone, R. Folate Metabolism in Malaria. *Bull. World Health Organ.* **1977,** *55,* 291-298.

22. Carrington, H. C.; Crowther, A. F.; Davey, D. G.; Levi, A. A.; Rose, F. L. A Metabolite of 'Paludrine' with High Antimalarial Activity. *Nature* **1951,** *168,* 1080-1080.

23. Falco, E. A.; Goodwin, L. G.; Hitchings, G. H.; Rollo, I. M.; Russell, P. B. 2:4-Diaminopyrimidines-A New Series of Antimalarials. *Br. J. Pharmacol. Chemother.* **1951,** *6,* 185-200.

24. Peterson, D. S.; Milhous, W. K.; Wellems, T. E. Molecular Basis of Differential Resistance to Cycloguanil and Pyrimethamine in *Plasmodium Falciparum* Malaria. *Proc. Natl. Acad. Sci.* **1990,** *87,* 3018.

25. Canfield, C. J.; Milhous, W. K.; Ager, A. L.; Rossan, R. N.; Sweeney, T. R.; Lewis, N. J.; Jacobus, D. P. PS-15: a Potent, Orally Active Antimalarial from a New Class of Folic Acid Antagonists. *Am. J. Trop. Med. Hyg.* **1993,** *49,* 121-126.

26. Childs, G. E.; Lambros, C. Analogues of N-Benzyloxydihydrotriazines: In Vitro Antimalarial Activity Against *Plasmodium falciparum*. *Ann. Trop. Med. Parasitol.* **1986,** *80,* 177-181.

27. Yuthavong, Y.; Tarnchompoo, B.; Vilaivan, T.; Chitnumsub, P.; Kamchonwongpaisan, S.; Charman, S. A.; McLennan, D. N.; White, K. L.; Vivas, L.; Bongard, E.; Thongphanchang, C.; Taweechai, S.; Vanichtanankul, J.; Rattanajak, R.; Arwon, U.; Fantauzzi, P.; Yuvaniyama, J.; Charman, W. N.; Matthews, D. Malarial Dihydrofolate Reductase as a Paradigm for Drug Development Against a Resistance-Compromised Target. *Proc. Natl. Acad. Sci.* **2012,** *109,* 16823-16828.

28. Medicines for Malaria Venture Home Page. https://www.mmv.org/ (accessed May 9, 2019).

29. Scott, D. A.; Coombs, G. H.; Sanderson, B. E. Effects of Methotrexate and Other Antifolates on the Growth and Dihydrofolate Reductase Activity of Leishmania Promastigotes. *Biochem. Pharmacol.* **1987,** *36,* 2043-2045.

30. Roth, B.; Aig, E.; Lane, K.; Rauckman, B. S. 2,4-Diamino-5-Benzylpyrimidines as

Antibacterial Agents. 4. 6-Substituted Trimethoprim Derivatives from Phenolic Mannich Intermediates. Application to The Synthesis of Trimethoprim and 3,5-Dialkylbenzyl Analogs. *J. Med. Chem.* **1980,** *23,* 535-541.

31. Zhao, Y.-F.; Liu, Z.-J.; Zhai, X.; Ge, D.-D.; Huang, Q.; Gong, P. Synthesis and In Vitro Antitumor Activity of Novel Diaryl Urea Derivatives. *Chin. Chem. Lett.* **2013,** *24,* 386-388.
32. Behrman, E. J. The Ortho-Para Ratio and the Intermediate in the Persulfate Oxidation of Aromatic Amines (The Boyland-Sims Oxidation). *J. Org. Chem.* **1992,** *57,* 2266-2270.
33. Behrman, E. J. The Persulfate Oxidation of Phenols and Arylamines (The Elbs and the Boyland–Sims Oxidations). *Org. React.* **1988,** *35,* 421-511.
34. Behrman, E. J. The Elbs and Boyland-Sims Peroxydisulfate Oxidations. *Beilstein J. Org. Chem.* **2006,** *2,* 22.
35. Hull, R. Pyrimidines. Part I. The Synthesis of Some 5-Hydroxypyrimidines. *J. Chem. Soc.* **1956,** 2033-2035.
36. McCall, J. M.; Tenbrink, R. E. Heterocyclic Chemistry: Benzoyl Peroxide Oxidation of Pyrimidines. *Synthesis* **1975,** *7,* 443-444.
37. Cabaj, J. E.; Wuts, P. G. M.; Henegar, K. E. Bromine-Mediated Addition of Nucleophiles to the Electron-Rich Pyrimidine Subunit of Tirilazad. *J. Org. Chem.* **1994,** *59,* 5090-5092.
38. Kovacic, P.; Reid, C. G.; Brittain, M. J. Aromatic Oxygenation. XII. Aromatic Oxygenation with Benzoyl Peroxide-Iodine. *J. Org. Chem.* **1970,** *35,* 2152-2156.
39. Yuan, C.; Liang, Y.; Hernandez, T.; Berriochoa, A.; Houk, K. N.; Siegel, D. Metal-Free Oxidation of Aromatic Carbon–Hydrogen Bonds Through a Reverse-Rebound Mechanism. *Nature* **2013,** *499,* 192.
40. Jing, L.; Wei, J.; Zhou, L.; Huang, Z.; Li, Z.; Zhou, X. Lithium Pipecolinate as a Facile and Efficient Ligand for Copper-Catalyzed Hydroxylation of Aryl Halides in Water. *Chem. Commun.* **2010,** *46,* 4767-4769.
41. Li, X.; Liu, Y. H.; Gu, W. J.; Li, B.; Chen, F. J.; Shi, B. F. Copper-Mediated Hydroxylation of Arenes and Heteroarenes Directed by a Removable Bidentate Auxiliary. *Org. Lett.* **2014,** *16,* 3904-3907.

42. Xia, G.; Li, Y.; Yang, Z.; Jiang, Z.-X. Development of a Scalable Process for **α**-Amino-**ω**-methoxyl-dodecaethylene Glycol. *Org. Process Res. Dev.* **2015,** *19,* 1769-1773.

43. Xia, S.; Gan, L.; Wang, K.; Li, Z.; Ma, D. Copper-Catalyzed Hydroxylation of (Hetero)aryl Halides under Mild Conditions. *J. Am. Chem. Soc.* **2016,** *138,* 13493-13496.

44. Richardson, M. L.; Stevens, M. F. G. Structural Studies on Bioactive Compounds. Part 37. Suzuki Coupling of Diaminopyrimidines: A New Synthesis of the Antimalarial Drug Pyrimethamine. *J. Chem. Res., Synop.* **2002,** 482-484.

45. Chang, D.; Zhu, D.; Shi, L. [3 + 2] Cycloadditions of Azides with Arynes via Photolysis of Phthaloyl Peroxide Derivatives. *J. Org. Chem.* **2015,** *80,* 5928-5933.

46. Powers, D. C.; Benitez, D.; Tkatchouk, E.; Goddard, W. A.; Ritter, T. Bimetallic Reductive Elimination from Dinuclear Pd(III) Complexes. *J. Am. Chem. Soc.* **2010,** *132,* 14092-14103.

47. Delia, T. J.; Venton, D. L. Pyrimidine *N*-Oxides. Preparation of 6-Chloro-2,4-diaminopyrimidine 3-*N*-oxide and Its Reactions. *J. Heterocycl. Chem.* **1972,** *9,* 73-75.

48. El-Ghomari, K.; Gorrod, J. W. Metabolic N-Oxygenation of 2,4-Diamino-6-substituted Pyrimidines. *Eur. J. Drug. Metab. Pharmacokinet.* **1987,** *12,* 253-258.

49. Balicki, R.; Cybulski, M.; Maciejewski, G. An Efficient Deoxygenation of Heteroaromatic N-Oxides Using Zinc Dust/Ammonium Formate Reagent System. *Synth. Commun.* **2003,** *33,* 4137-4141.

50. Gupta, S.; Sureshbabu, P.; Singh, A. K.; Sabiah, S.; Kandasamy, J. Deoxygenation of Tertiary Amine N-oxides Under Metal Free Condition Using Phenylboronic Acid. *Tetrahedron Lett.* **2017,** *58,* 909-913.

51. Benson, S. W. Bond Energies. *J. Chem. Educ.* **1965,** *42,* 502.

52. Shah, P.; Westwell, A. D. The Role of Fluorine in Medicinal Chemistry. *J. Enzyme Inhib. Med. Chem.* **2007,** *22,* 527-540.

APPENDIX

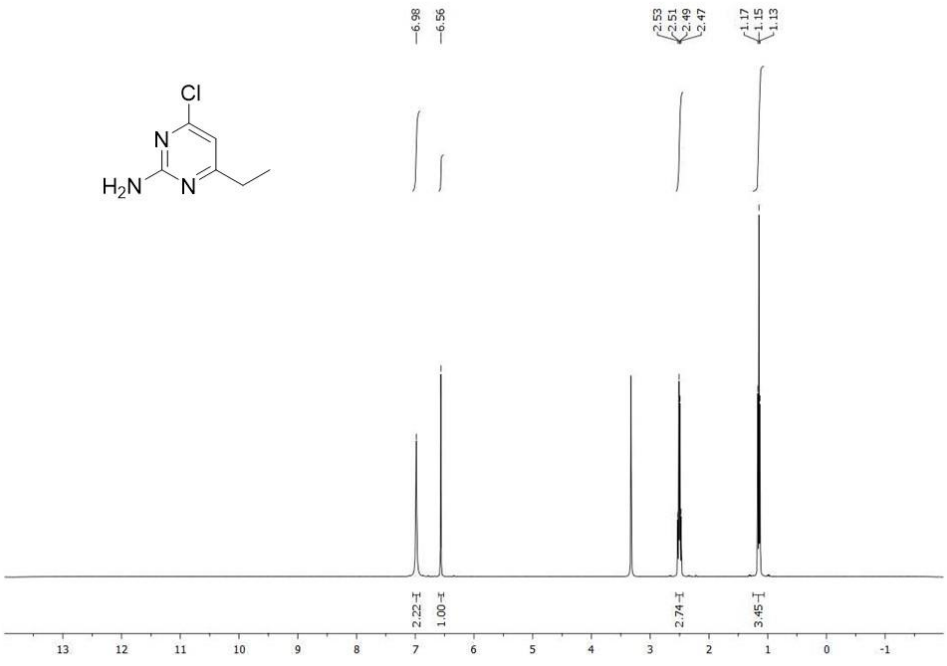


Figure A-1 ^{1}H NMR spectrum of 2-amino-4-chloro-6-ethylpyrimidine.

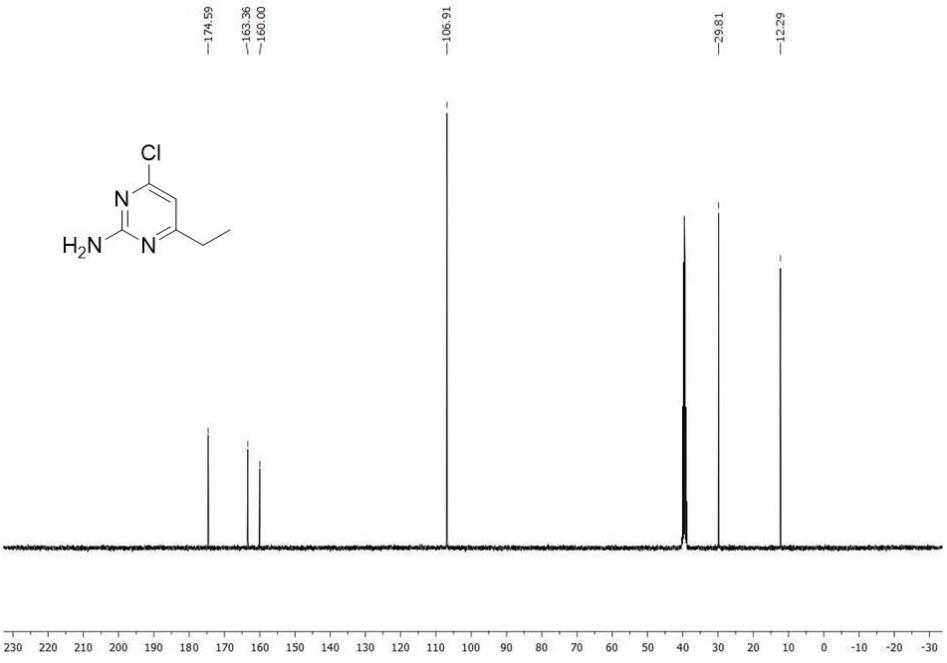


Figure A-2 ^{13}C NMR spectrum of 2-amino-4-chloro-6-ethylpyrimidine.

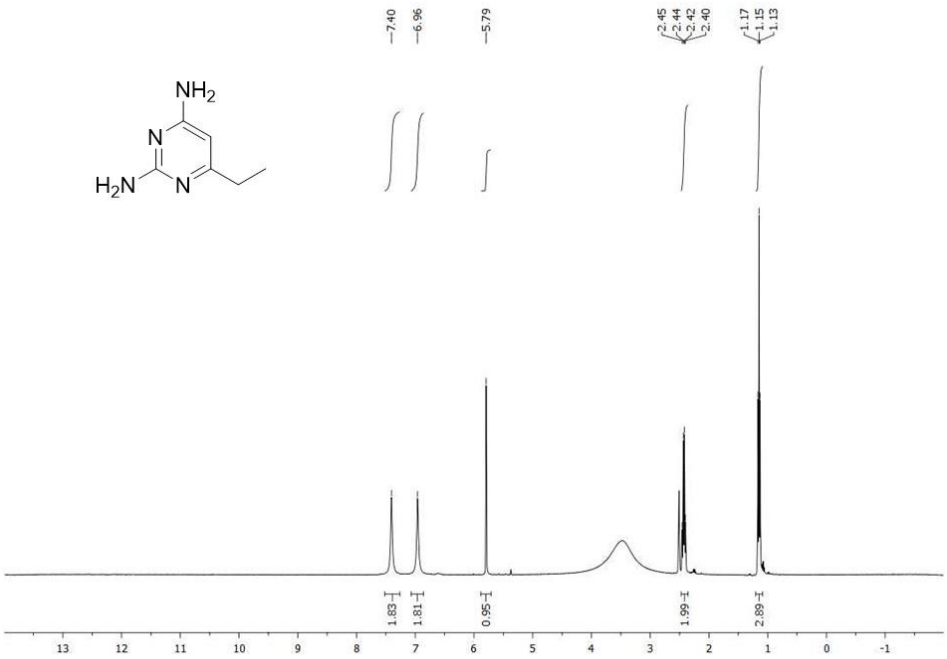


Figure A-3 ^{1}H NMR spectrum of 2,4-diamino-6-ehthylpyrimidine (**1a**).

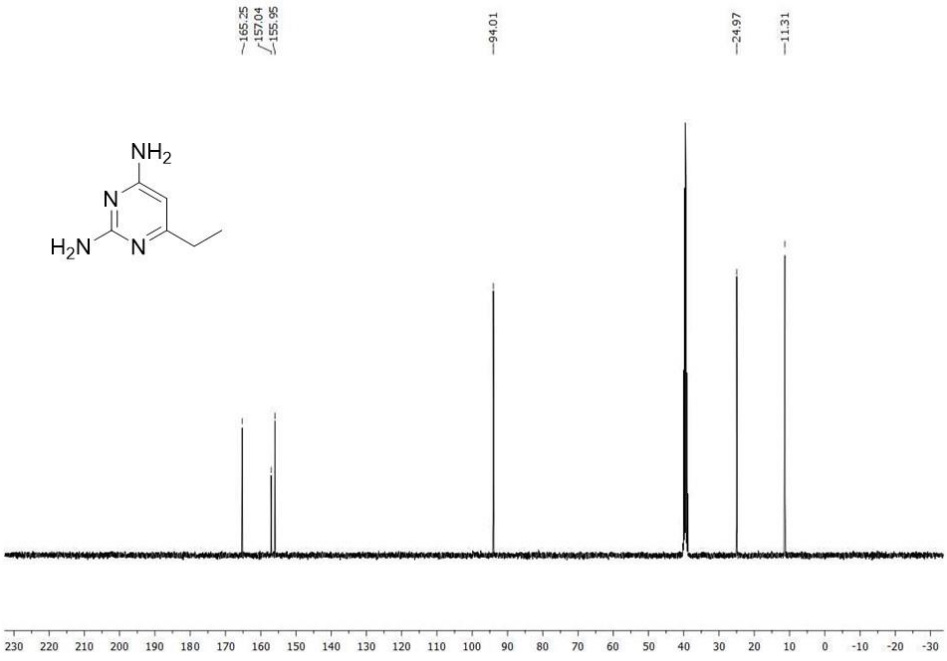


Figure A-4 ^{13}C NMR spectrum of 2,4-diamino-6-ehthylpyrimidine (**1a**).

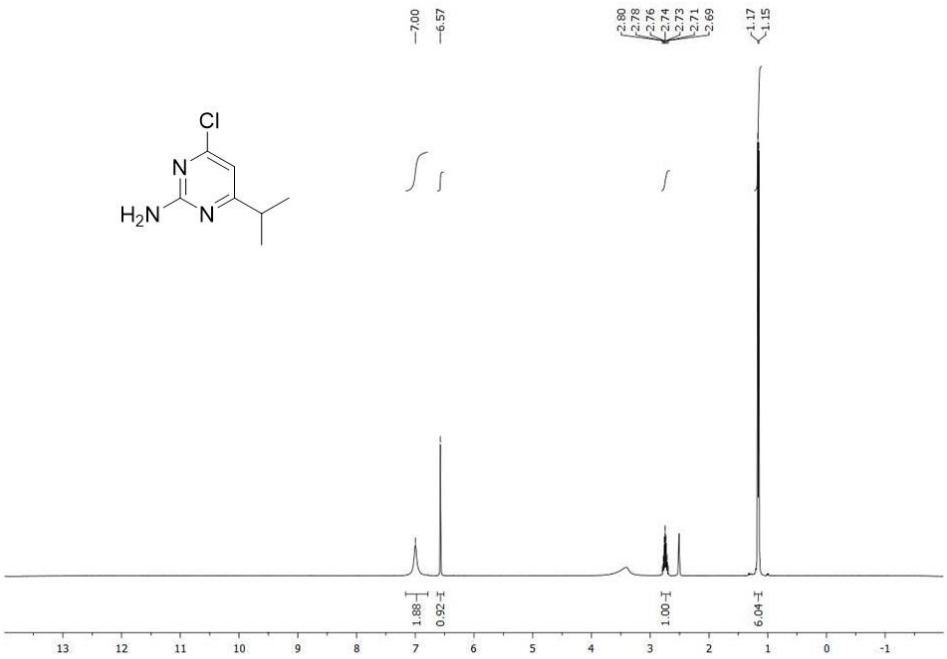


Figure A-5 ^{1}H NMR spectrum of 2-amino-4-chloro-6-isopropylpyrimidine.

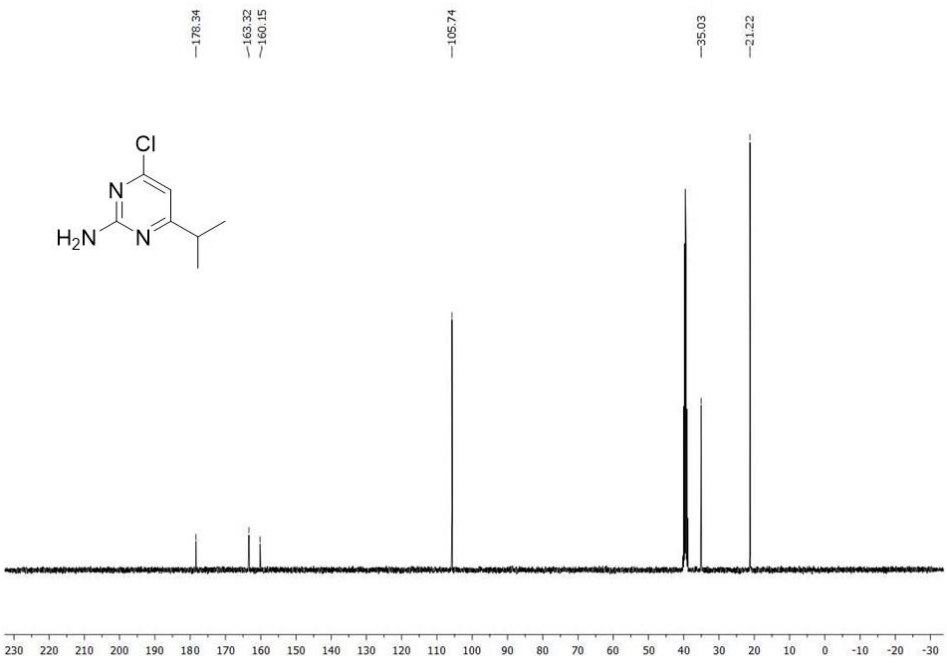


Figure A-6 ^{13}C NMR spectrum of 2-amino-4-chloro-6-isopropylpyrimidine.

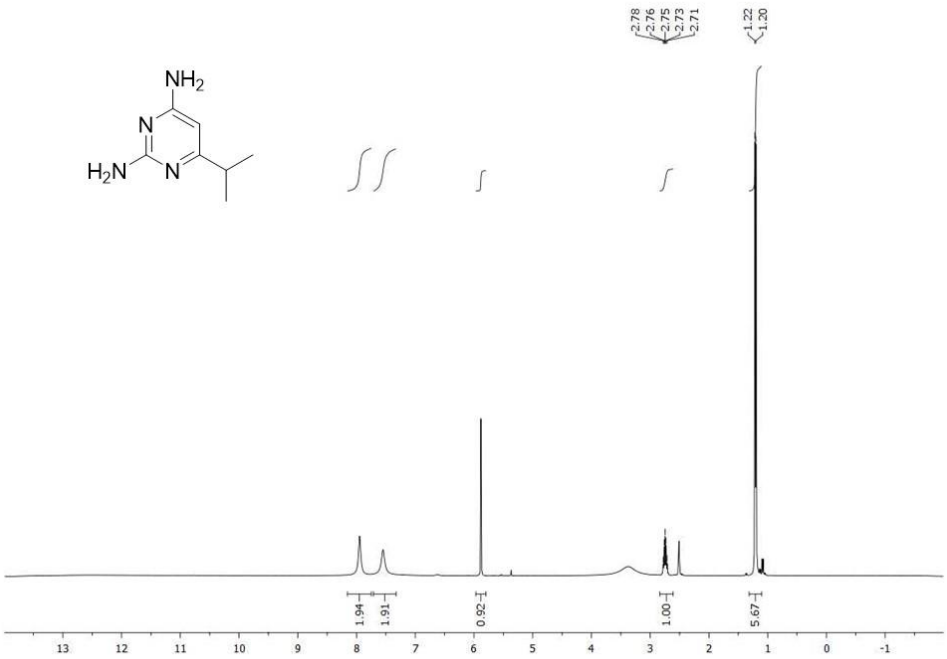


Figure A-7 ^{1}H NMR spectrum of 2,4-diamino-6-isopropylpyrimidine (**2a**).

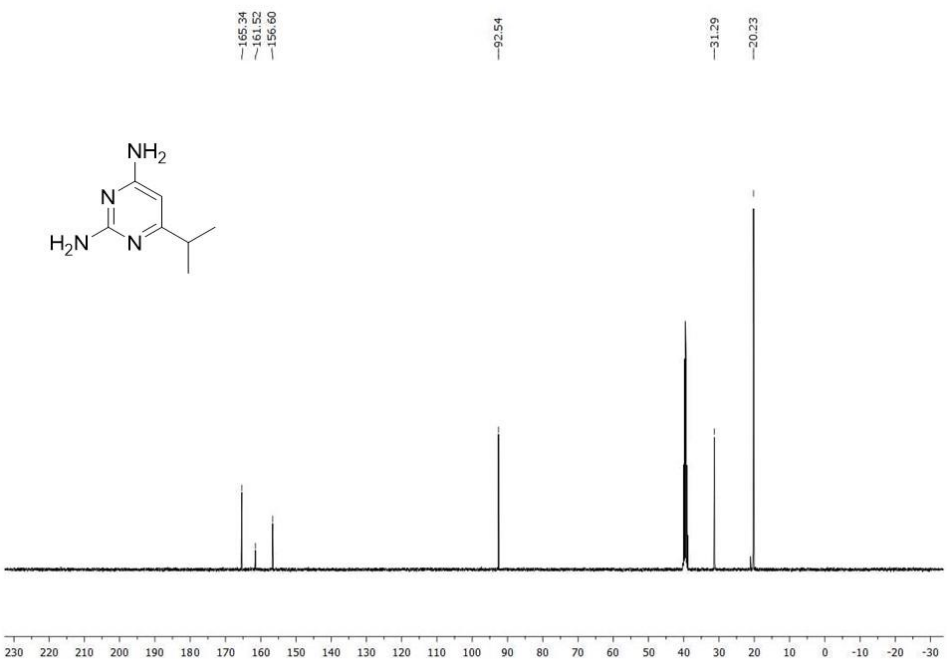


Figure A-8 ^{13}C NMR spectrum of 2,4-diamino-6-isopropylpyrimidine (**2a**).

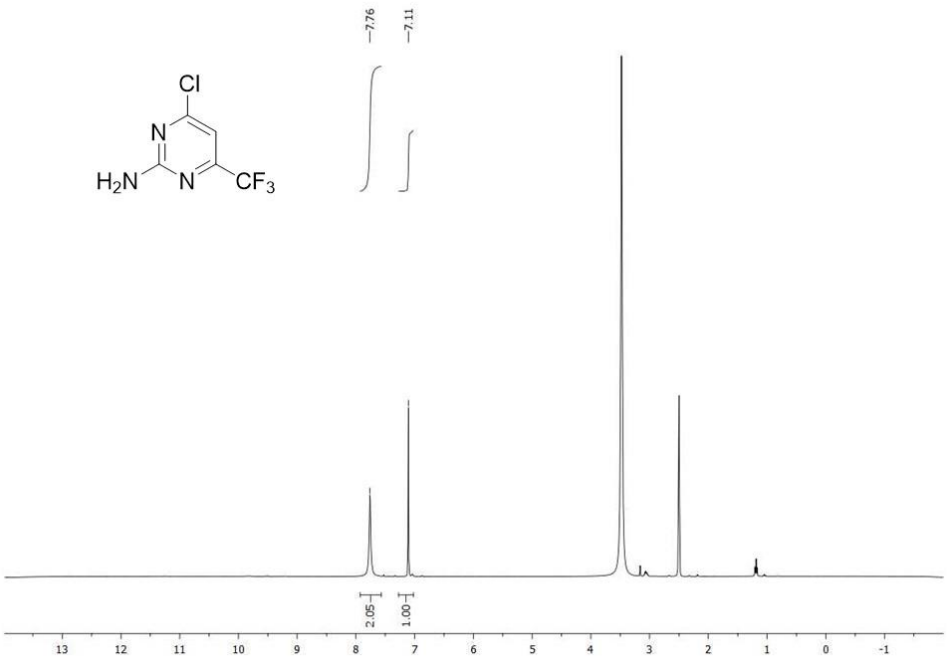


Figure A-9 ^{1}H NMR spectrum of 2-amino-4-chloro-6-trifluoromethylpyrimidine.

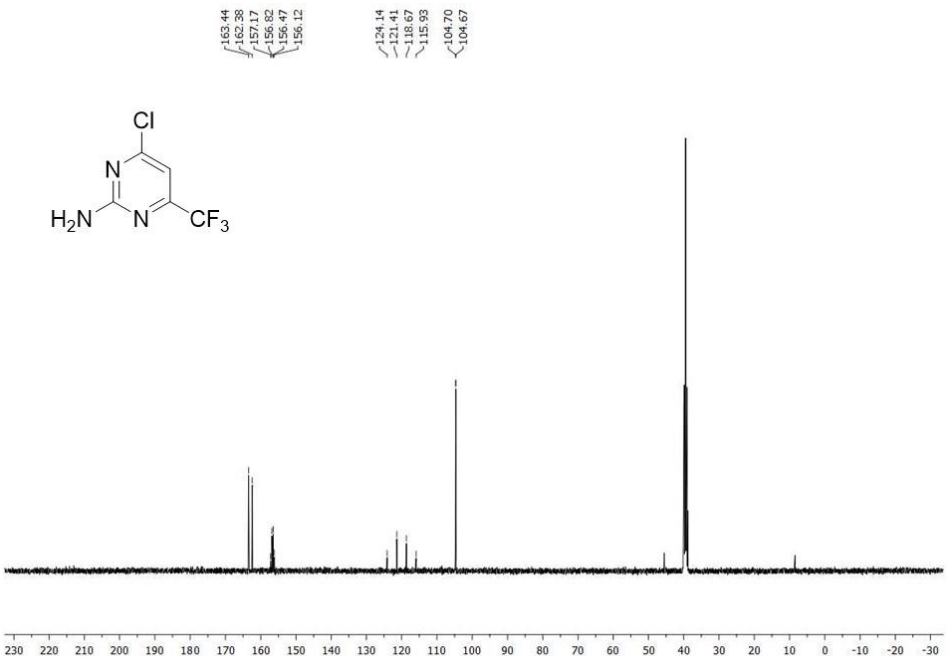


Figure A-10 ^{13}C NMR spectrum of 2-amino-4-chloro-6-trifluoromethylpyrimidine.

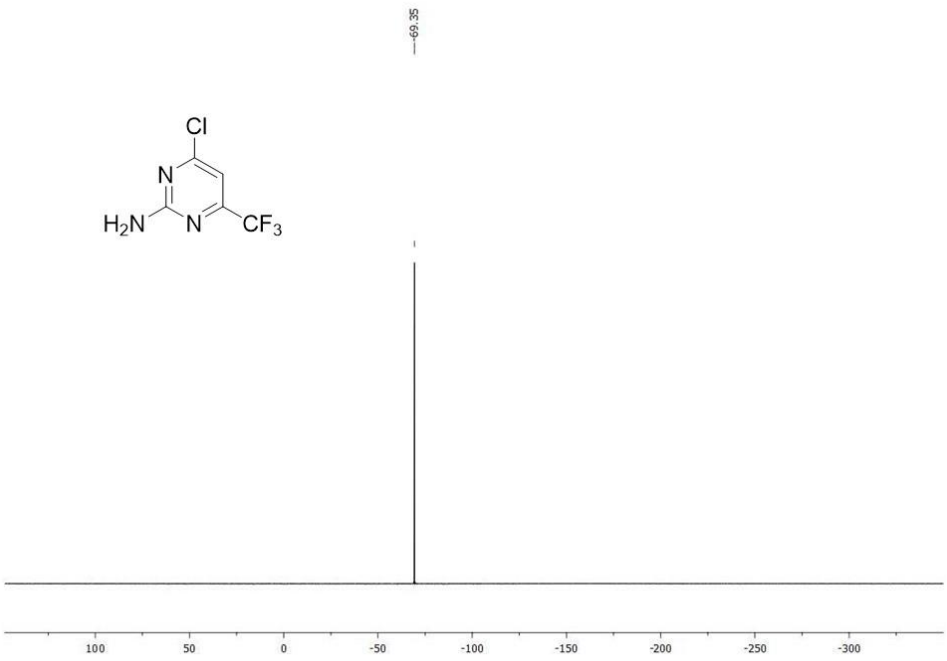


Figure A-11 ^{19}F NMR spectrum of 2-amino-4-chloro-6-trifluoromethylpyrimidine.

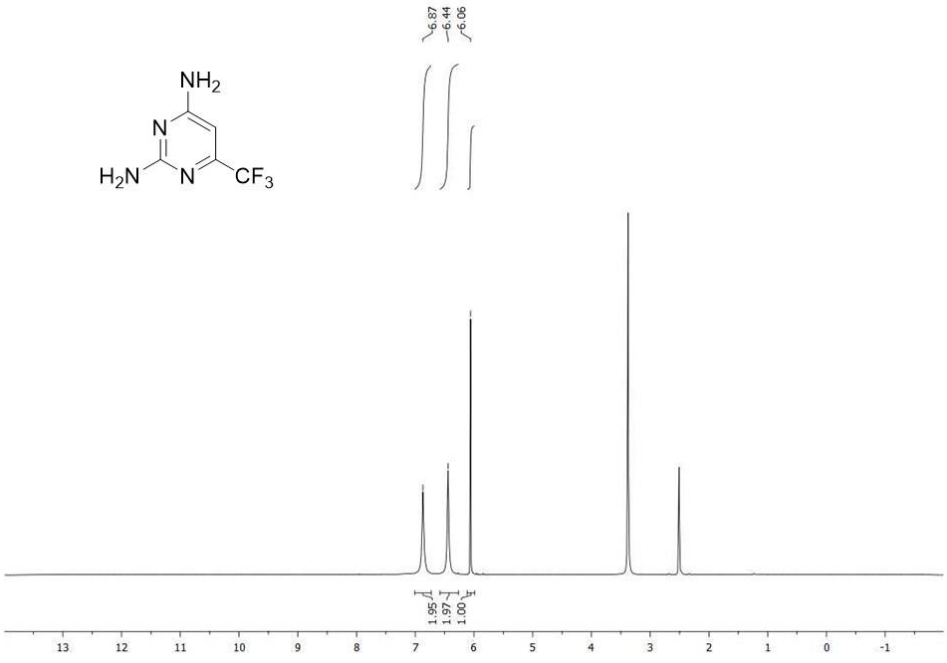


Figure A-12 ^{1}H NMR spectrum of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**).

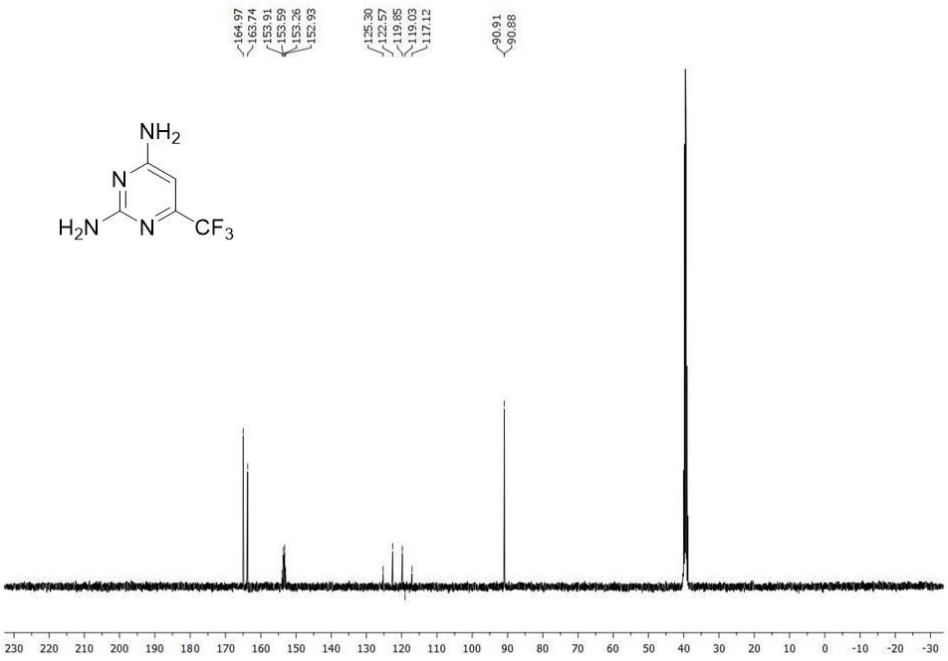


Figure A-13 ^{13}C NMR spectrum of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**).

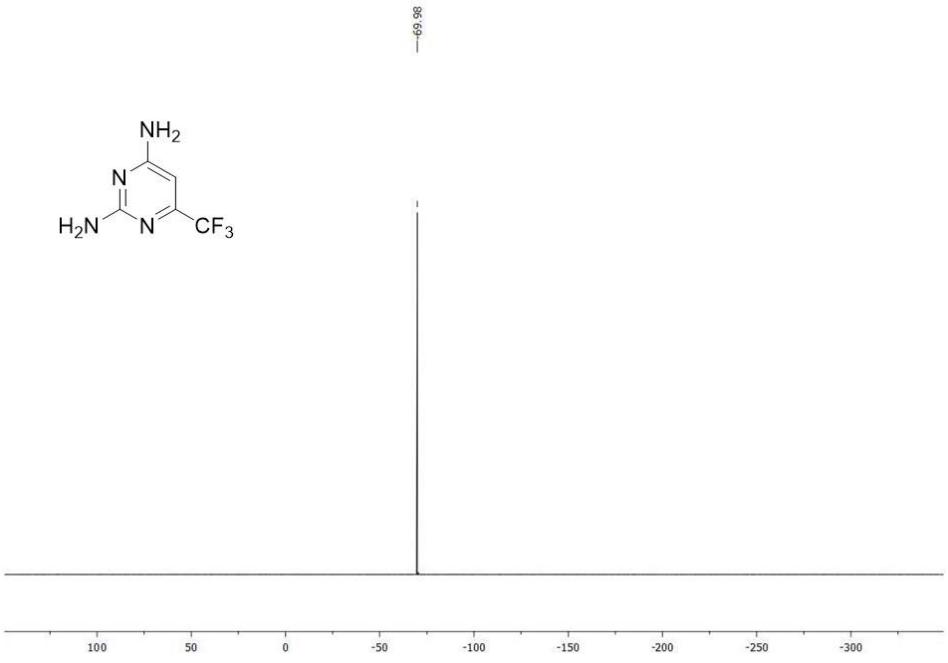


Figure A-14 ^{19}F NMR spectrum of 2,4-diamino-6-trifluoromethylpyrimidine (**4a**).

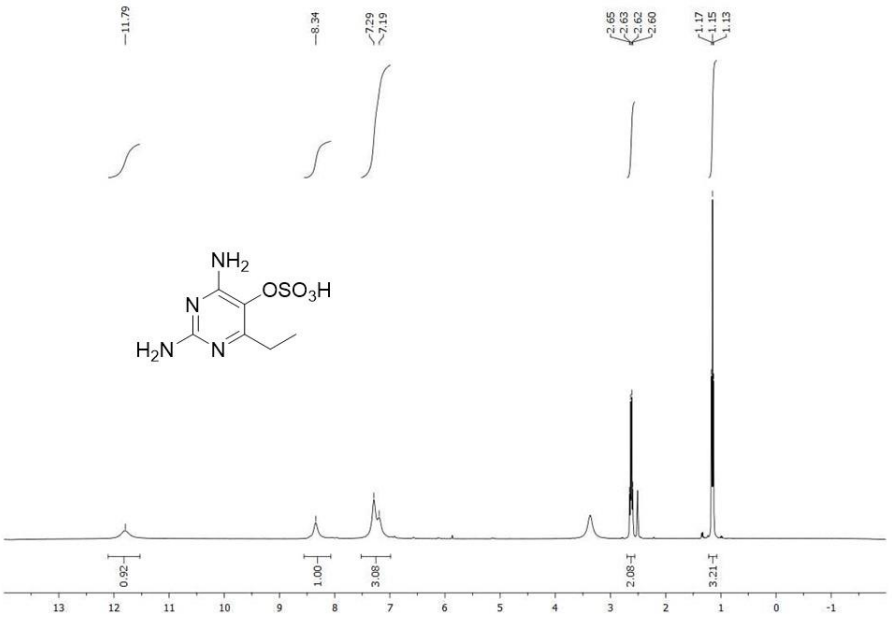


Figure A-15 ^{1}H NMR spectrum of 2,4-diamino-6-ehthyl-5-pyrimidyl hydrogen sulfate (**1b**).

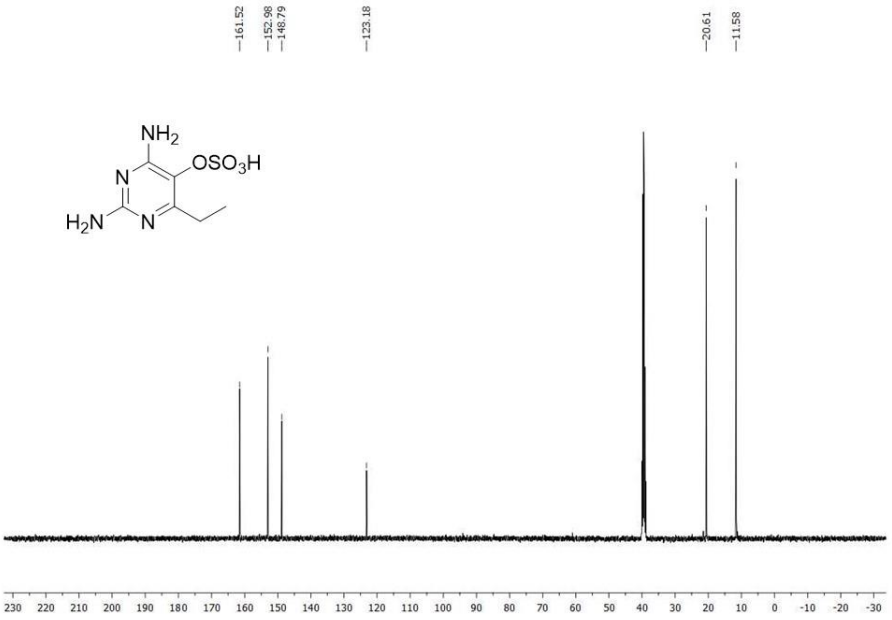


Figure A-16 ^{13}C NMR spectrum of 2,4-diamino-6-ehthyl-5-pyrimidyl hydrogen sulfate (**1b**).

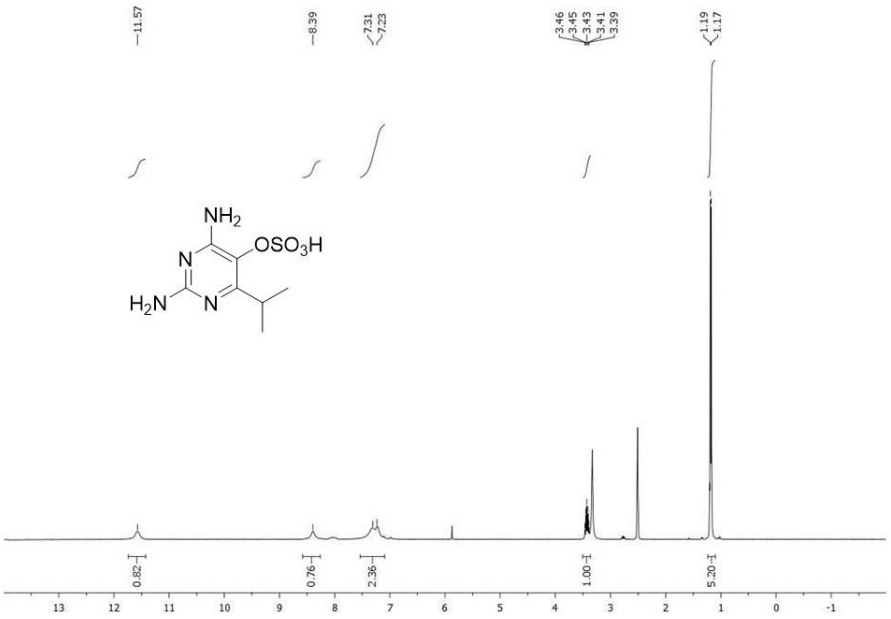


Figure A-17 ^{1}H NMR spectrum of of 2,4-diamino-6-isopropyl-5-pyrimidinyl hydrogen sulfate (**2b**).

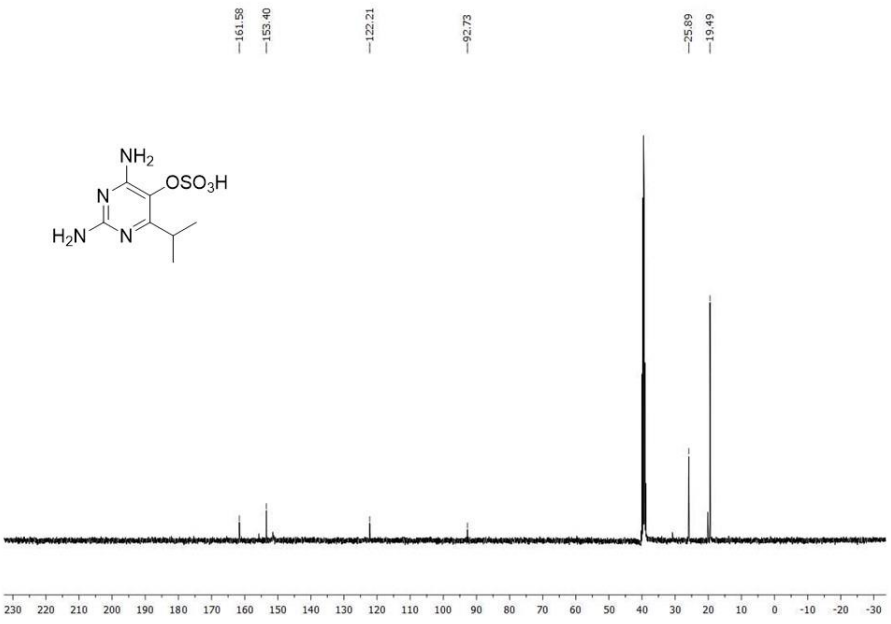


Figure A-18 ^{13}C NMR spectrum of of 2,4-diamino-6-isopropyl-5-pyrimidinyl hydrogen sulfate (**2b**).

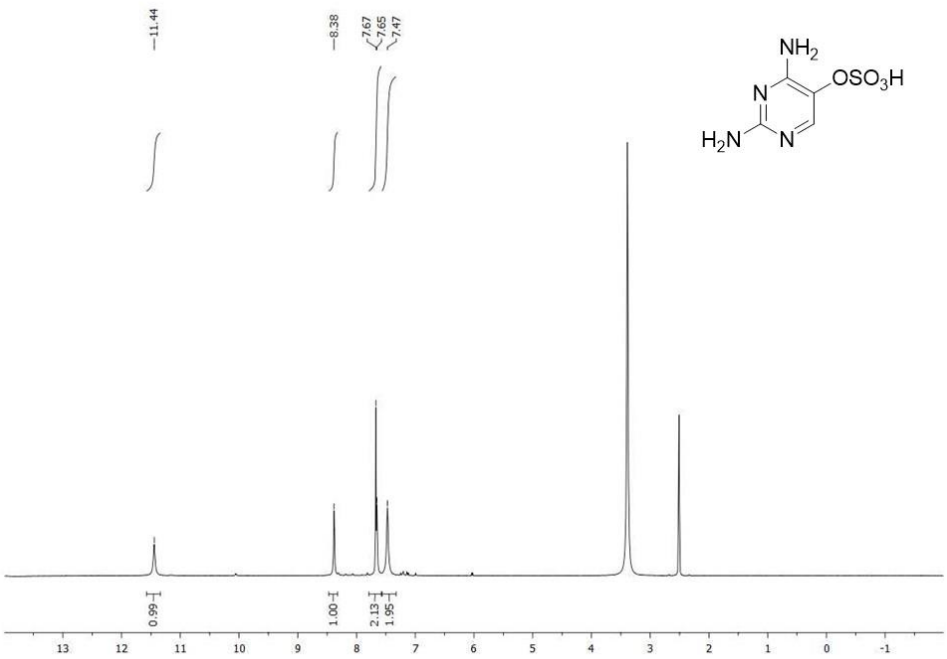


Figure A-19 ^{1}H NMR spectrum of 2,4-diamino-5-pyrimidinyl hydrogen sulfate (**3b**).

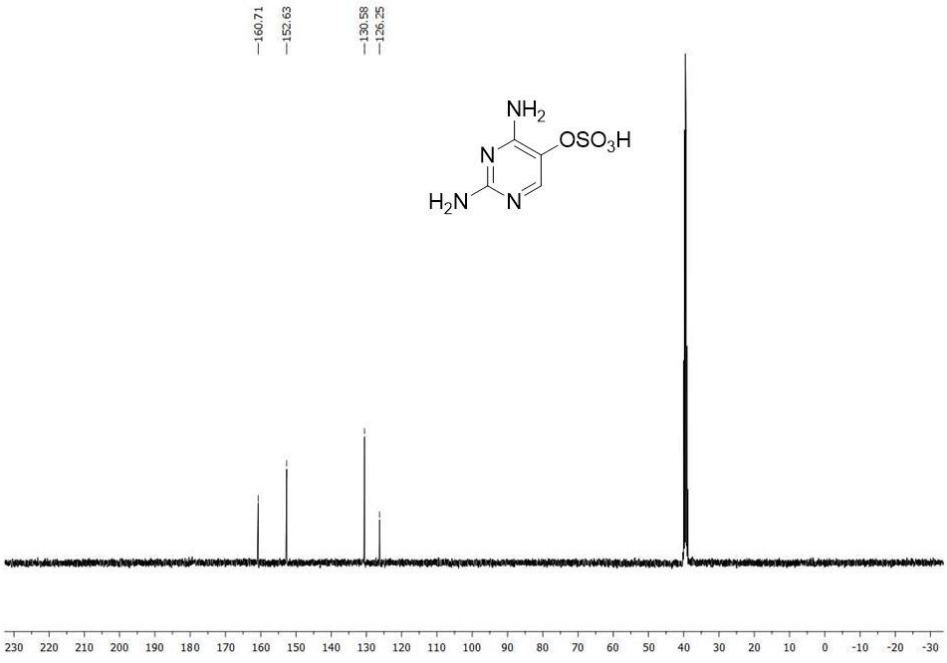


Figure A-20 ^{13}C NMR spectrum of 2,4-diamino-5-pyrimidinyl hydrogen sulfate (**3b**).

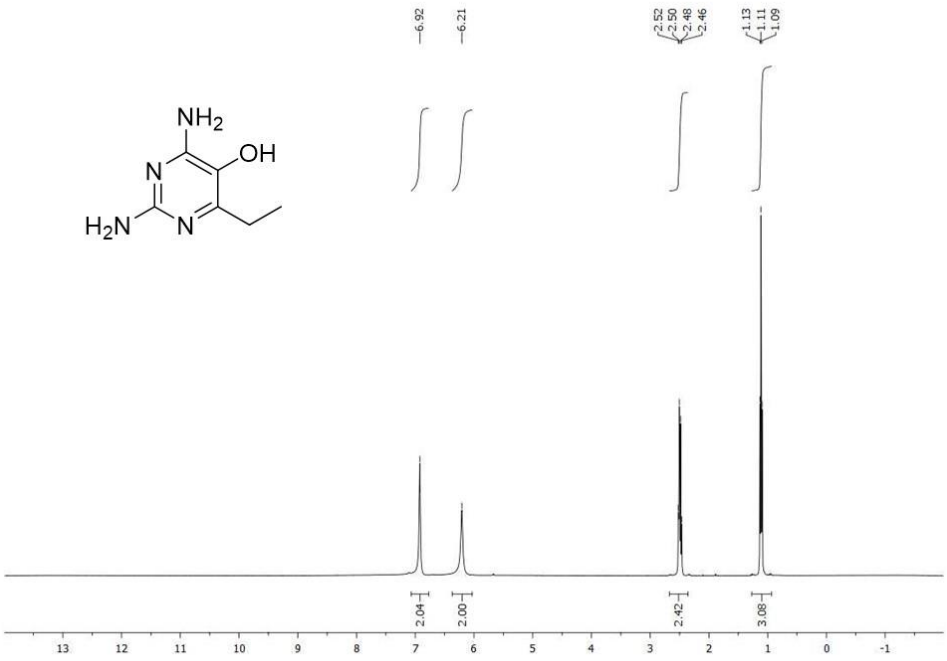


Figure A-21 ^{1}H NMR spectrum of 2,4-diamino-5-hydroxy-6-ethylpyrimidine (**1c**).

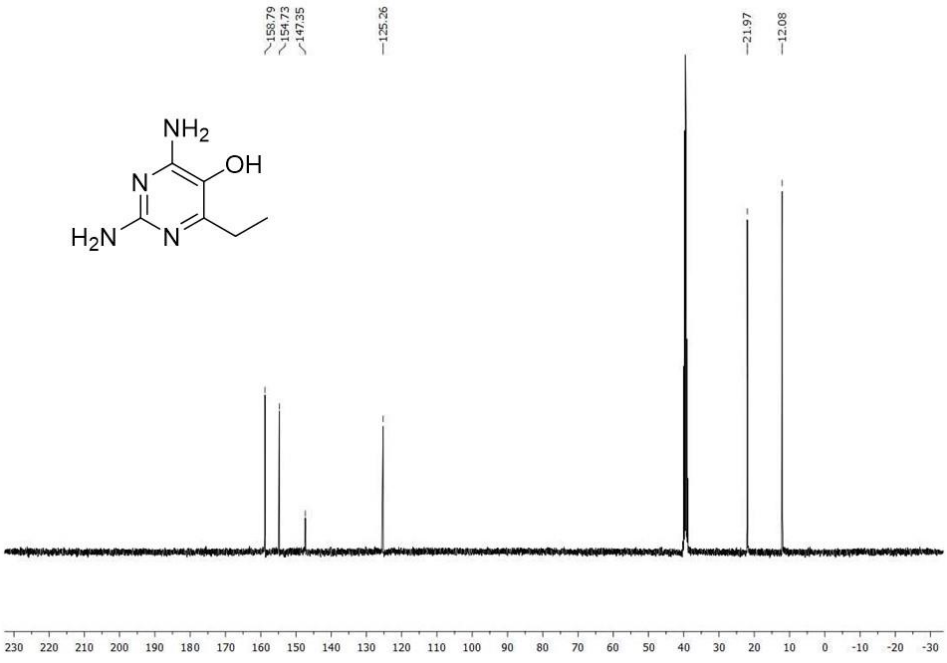


Figure A-22 ^{13}C NMR spectrum of 2,4-diamino-5-hydroxy-6-ethylpyrimidine (**1c**).

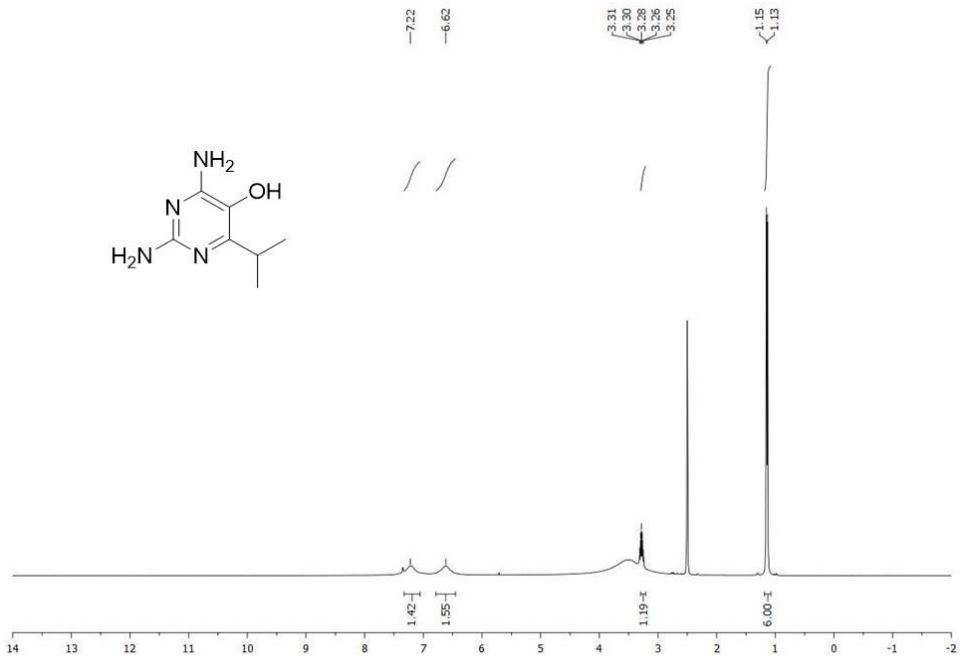


Figure A-23 ^{1}H NMR spectrum of 2,4-diamino-5-hydroxy-6-isopropylpyrimidine (**2c**).

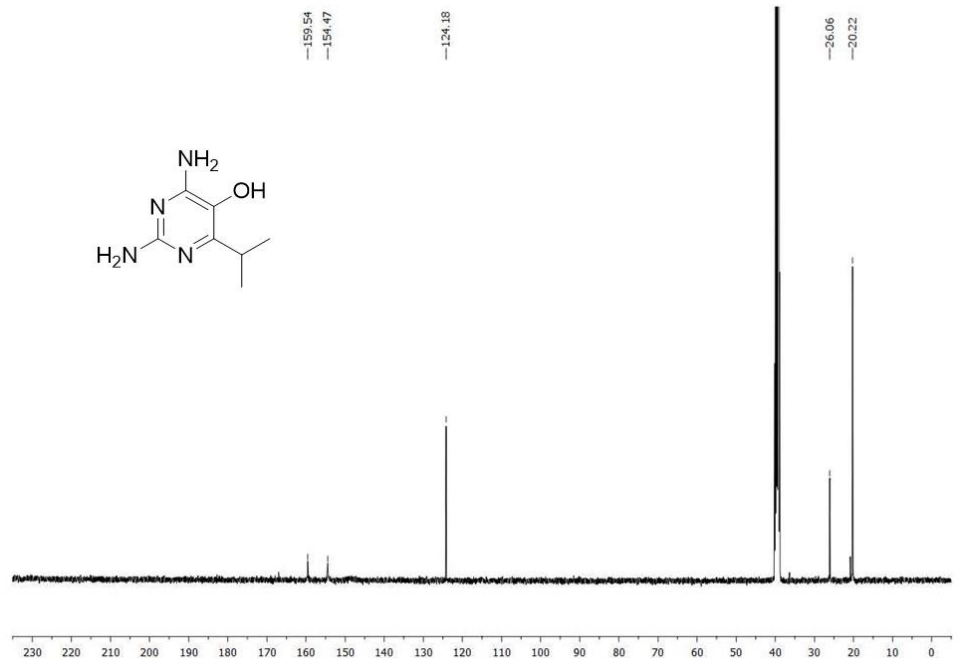


Figure A-24 ^{13}C NMR spectrum of 2,4-diamino-5-hydroxy-6-isopropylpyrimidine (**2c**).

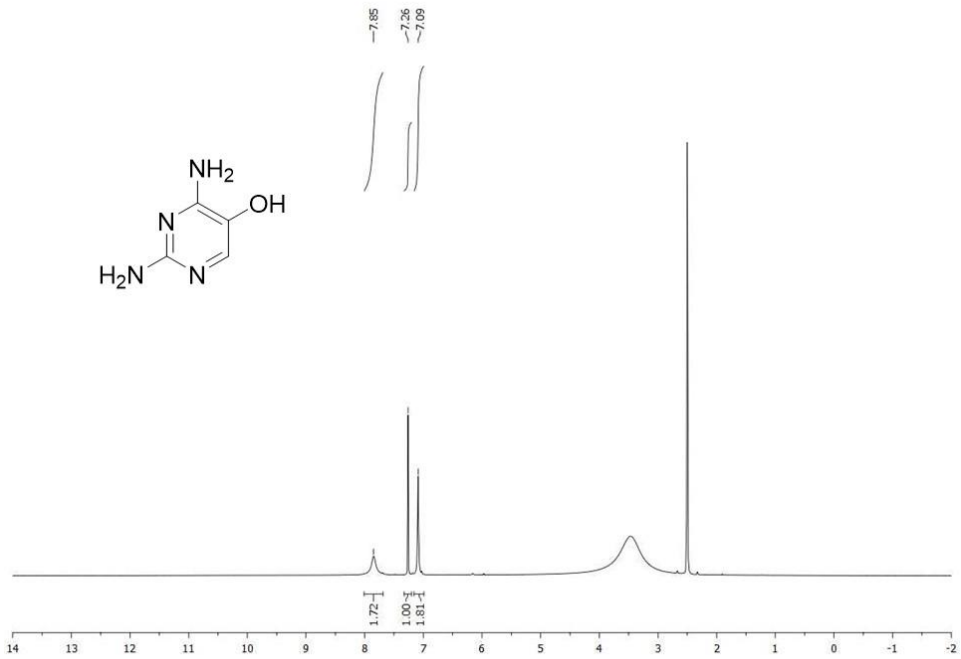


Figure A-25 ^{1}H NMR spectrum of 2,4-diamino-5-hydroxypyrimidine (**3c**).

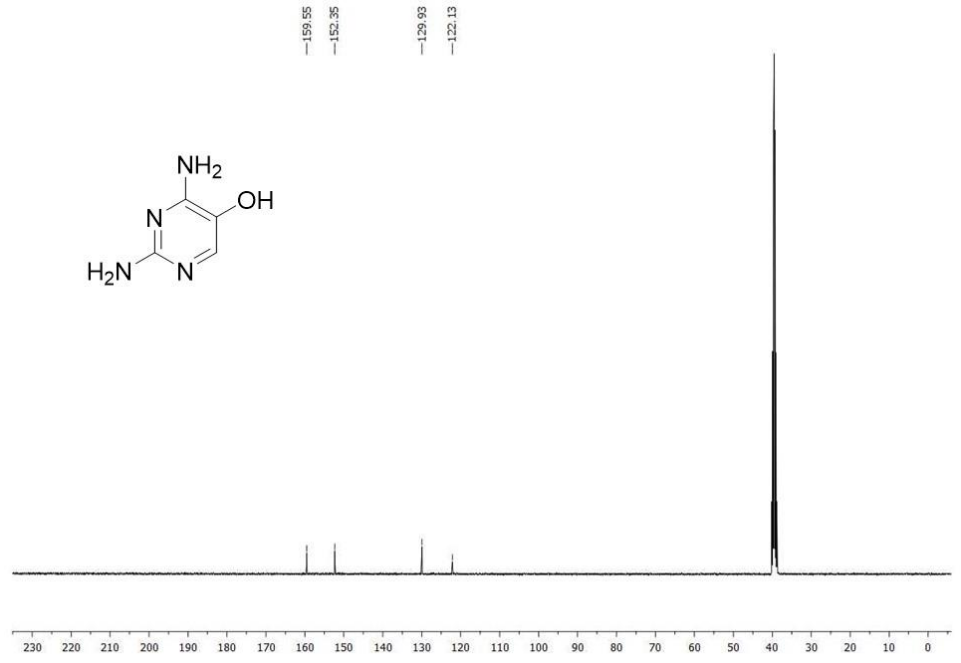


Figure A-26 ^{13}C NMR of 2,4-diamino-5-hydroxypyrimidine (**3c**).

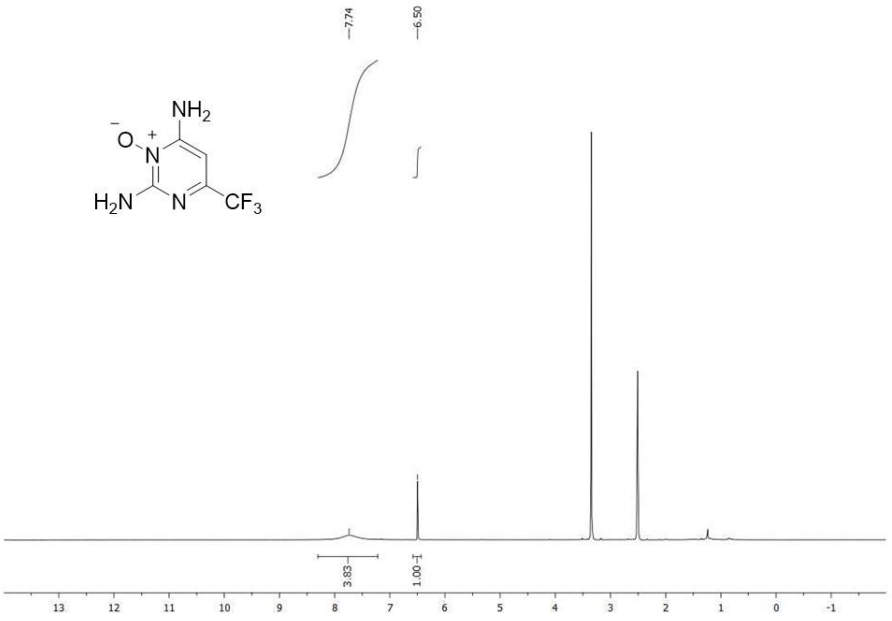


Figure A-27 ^{1}H NMR spectrum of 2,4-diamino-6-trifluoromethylpyrimidine-3-*N*-oxide (**4d**).

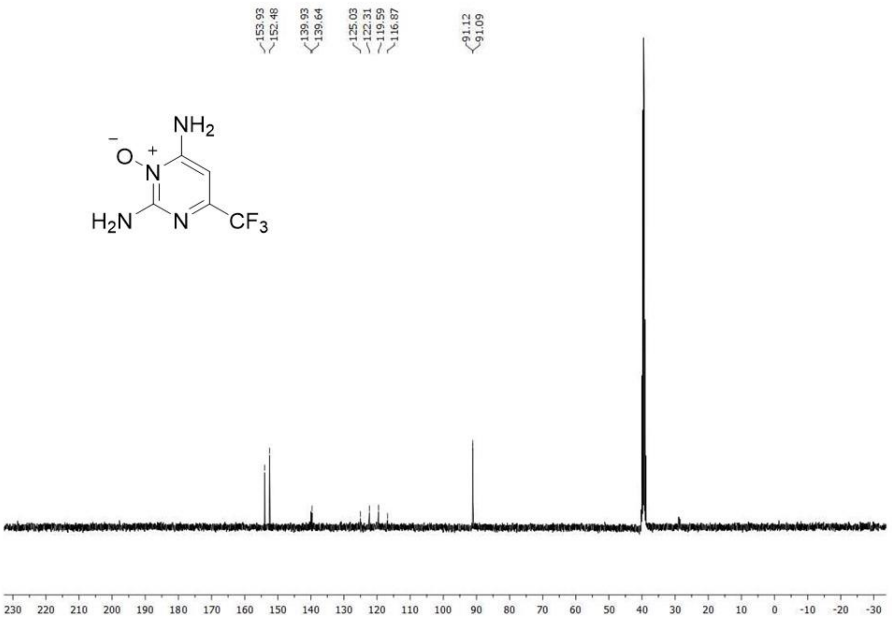


Figure A-28 ^{13}C NMR spectrum of 2,4-diamino-6-trifluoromethylpyrimidine-3-*N*-oxide (**4d**).

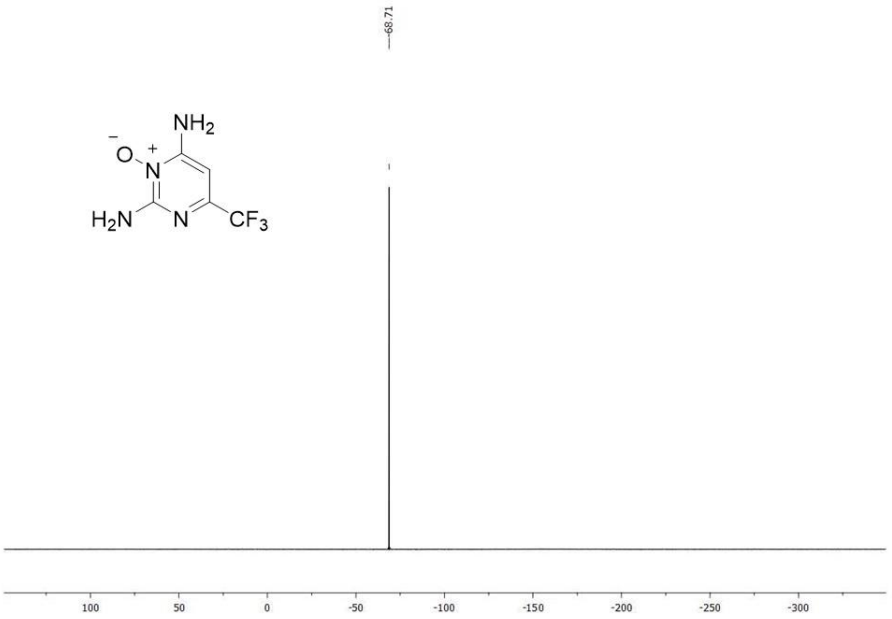


Figure A-29 ^{19}F NMR spectrum of 2,4-diamino-6-trifluoromethylpyrimidine-3-*N*-oxide (**4d**).

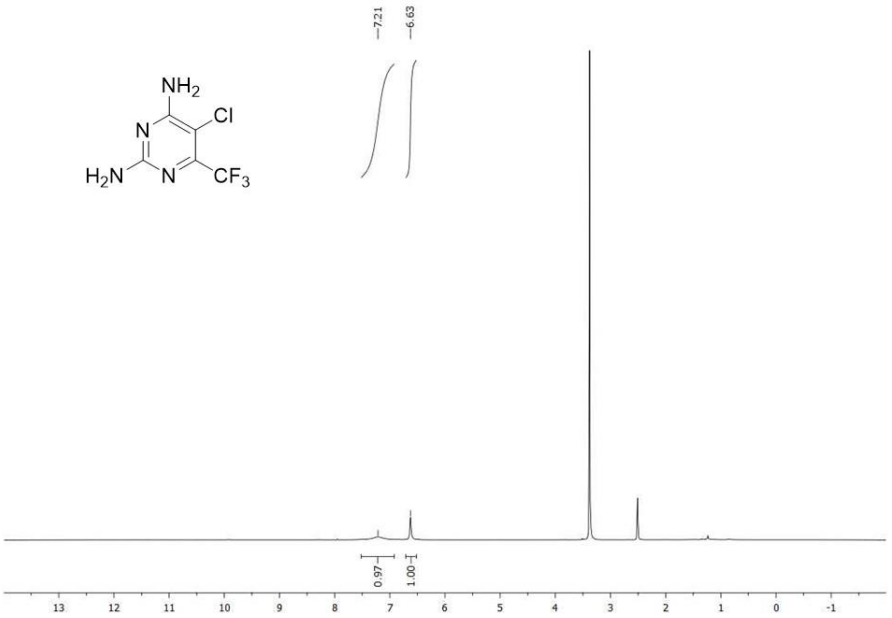


Figure A-30 ^{1}H NMR spectrum of 2,4-diamino-5-chloro-6-trifluoromethylpyrimidine (**4e**).

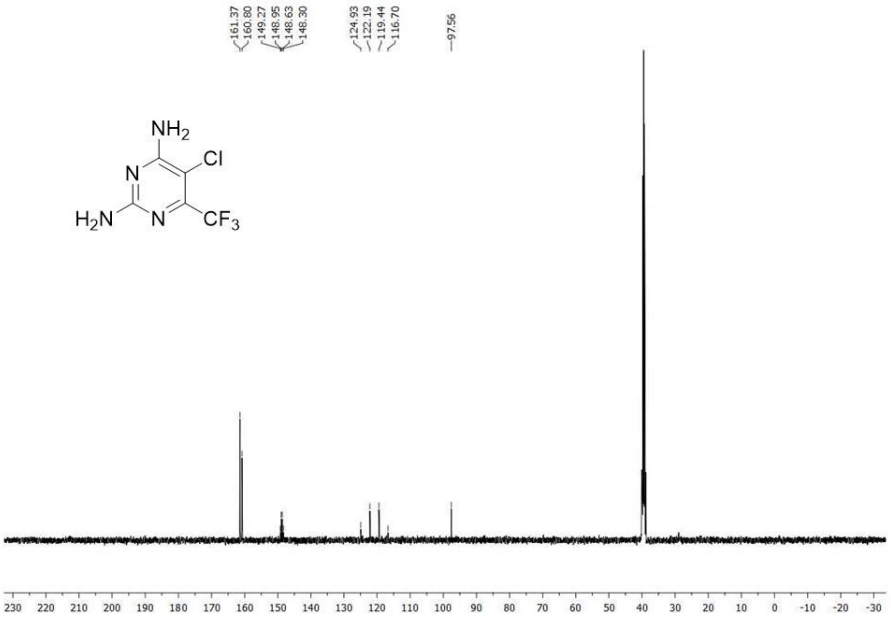


Figure A-31 ^{13}C NMR spectrum of 2,4-diamino-5-chloro-6-trifluoromethylpyrimidine (**4e**).

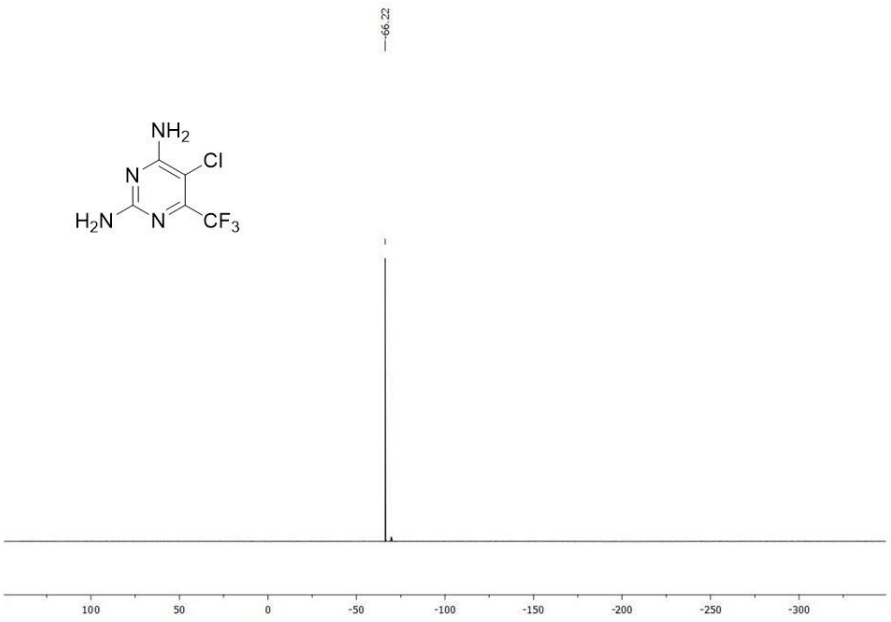


Figure A-32 ^{19}F NMR spectrum of 2,4-diamino-5-chloro-6-trifluoromethylpyrimidine (**4e**).

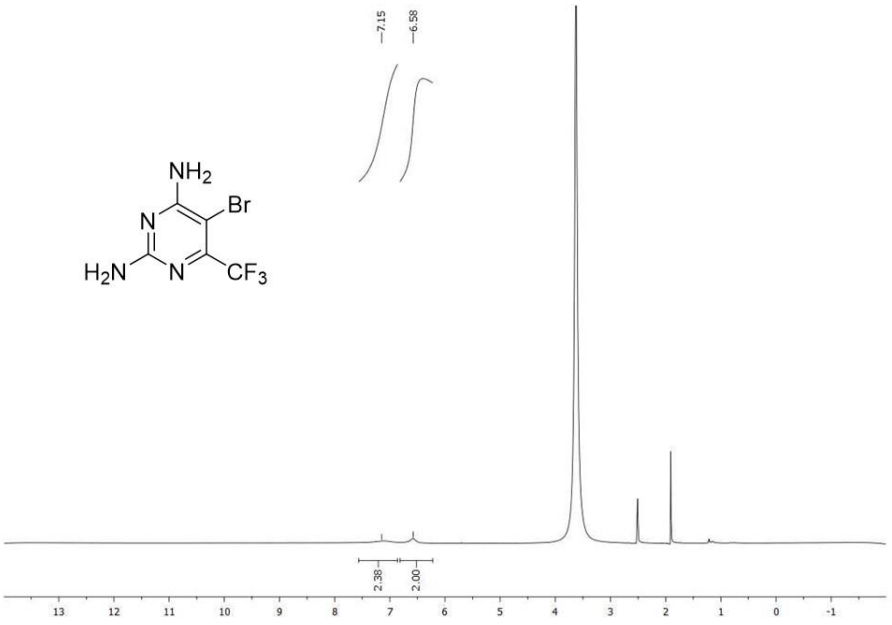


Figure A-33 ^{1}H NMR spectrum of 2,4-diamino-5-bromo-6-trifluoromethylpyrimidine (**4f**).

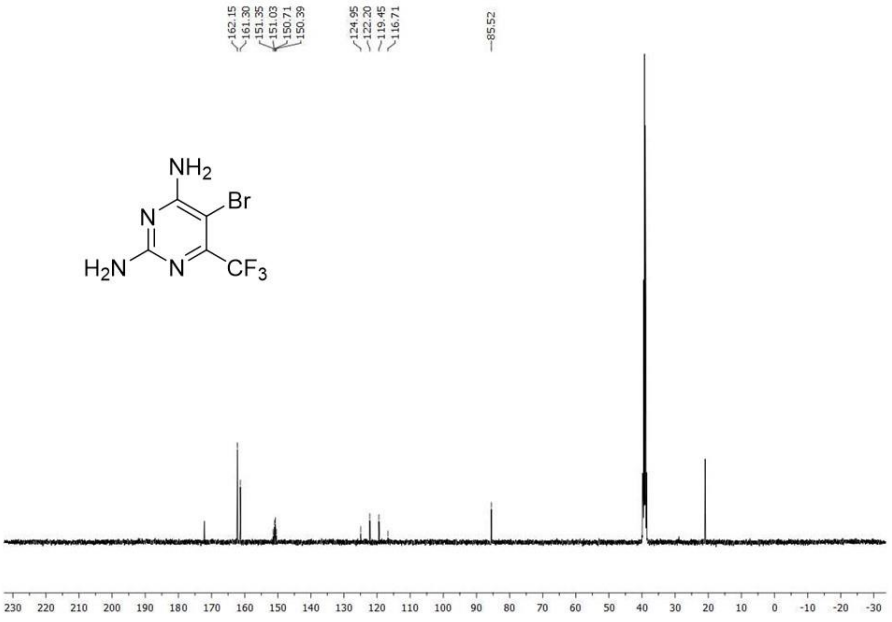


Figure A-34 ^{13}C NMR spectrum of 2,4-diamino-5-bromo-6-trifluoromethylpyrimidine (**4f**).

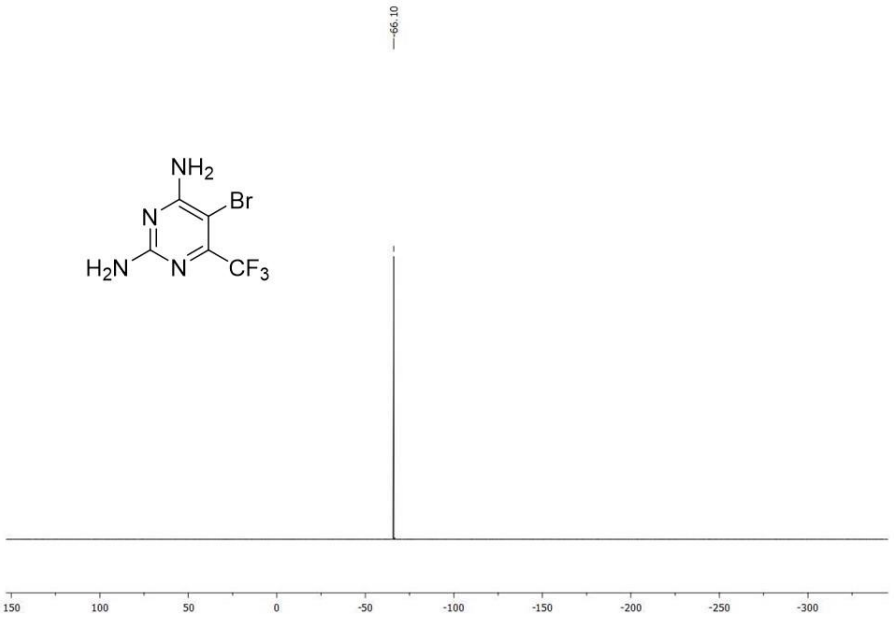


Figure A-35 ^{19}F NMR spectrum of 2,4-diamino-5-bromo-6-trifluoromethylpyrimidine (**4f**).

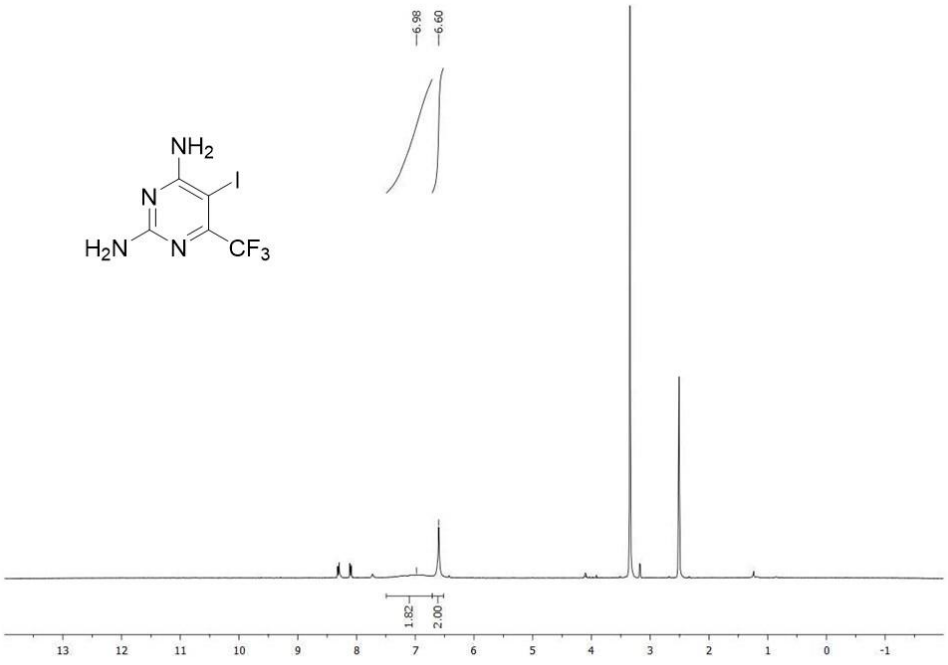


Figure A-36 ^{1}H NMR spectrum of 2,4-diamino-5-iodo-6-trifluoromethylpyrimidine (**4g**).

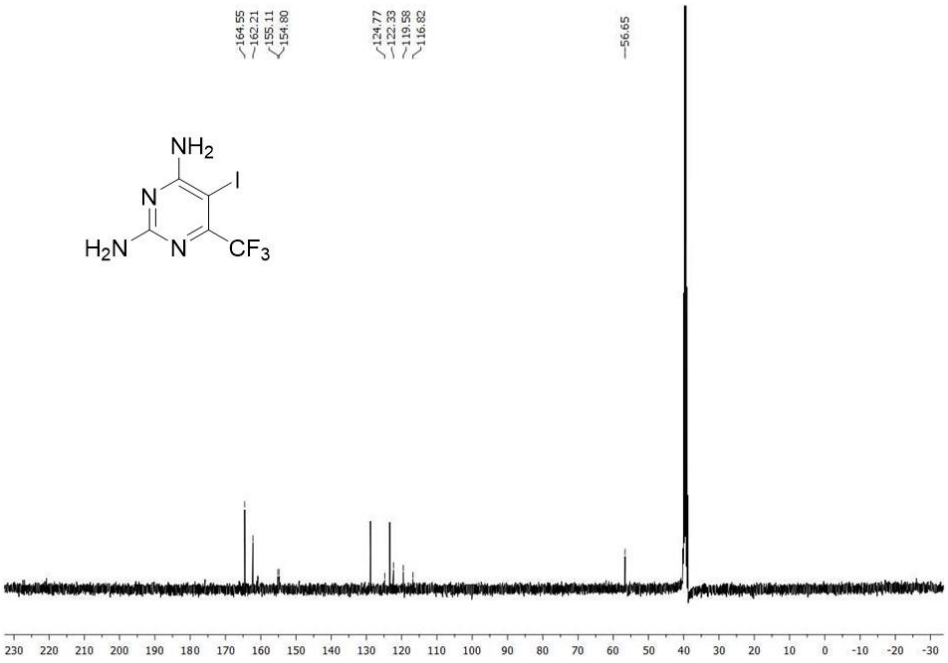


Figure A-37 ^{13}C NMR spectrum of 2,4-diamino-5-iodo-6-trifluoromethylpyrimidine (**4g**).

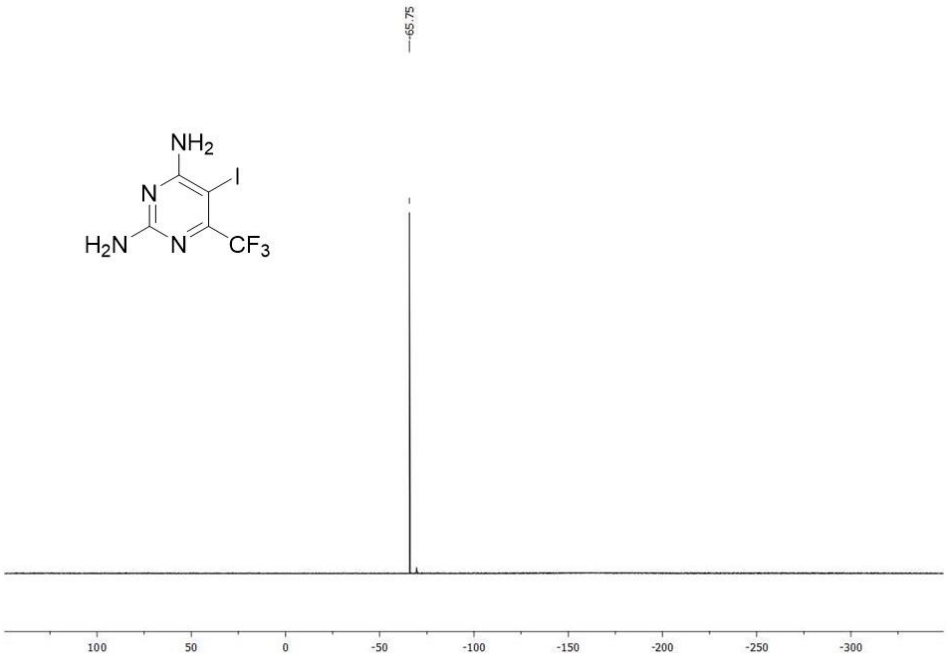


Figure A-38 ^{19}F NMR spectrum of 2,4-diamino-5-iodo-6-trifluoromethylpyrimidine (**4g**).

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