การสังเคราะห์สารอนุพันธ์ของไอโซไทอะโซลไพริมิดิน เพื่อใช้เป็นสารต้านจุลชีพ

นางสาว สุมนัส อัครภาสิชย์กร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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SYNTHESIS OF ISOTHIAZOLOPYRIMIDINE DERIVATIVES AS POTENTIAL ANTIMICROBIAL AGENTS

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Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requiredments for the Master's Degree.

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สุมนัส อัครภาฒิชย์กร : การสังเคราะห์สารอนุพันธ์ของไอโซไทอะโซลไพริมิดินเพื่อใช้เป็นสารต้านจุลชีพ (SYNTHESIS OF ISOTHIAZOLOPYRIMIDINE DERIVATIVES AS POTENTIAL ANTIMICROBIAL AGENTS) อ.ที่ปรึกษา: ผศ. ดร. ชานาญ ภัตรพานิช และ อาจารย์ สุวรรณา หวังวีรวงศ์, 114 หน้า.

งานวิจัยนี้มีจุดมุ่งหมายเพื่อสังเคราะห์สารประกอบอินทรีย์ตัวใหม่ ที่คาดว่ามีฤทธิ์ต้านจุลชีพ และ ศึกษากระบวนการสังเคราะห์สารประกอบอนุพันธ์ของไอโซไทอะโซลไพริมิดิน

การสังเคราะห์สารอนุพันธ์ของไอโซไทอะโซลไพริมิดินหลายตัว โดยเริ่มต้นจากอนุพันธ์ของ 3,5-ไดอะมิโนไอโซไทอะโซล ทำปฏิกริยากับอนุพันธ์ของ ไอโซไทโอไซยาเนท ทำให้ได้สารประกอบอนุพันธ์ เอ็น-ไอโซไทอะโซลิล ไทโอยูเรียซึ่งสามารถทำให้เป็นวงด้วยความร้อน จะได้ผลผลิต 8 ตัวเป็น 5-สับสะติติวเตด-3-สับสะติติวเตด อะมิโนไอโซไทอะโซล[3,4-ดี]ไพริมิดิน-4-โอน-6(7-เอช)-ไทโอน 4 ตัว และ 5,7-ไดสับสะติติวเตด-3-อะมิโน ไอโซไทอะโซล[5,4-ดี]ไพริมิดิน-4-อีมีน-6-ไทโอน 4 ตัว และ สารระหว่างกระบวนการอีก 8 ตัว ได้แก่ 3-อะมิโน-4-คาร์เบททอกซี-5-สับสะติติวเตด อะมิโนไอโซไทอะโซล 2 ตัว 3-อะมิโน-4-ไซยาโน-5-สับสะติติวเตด อะมิโนไอโซไทอะโซล 2 ตัว และ เอ็น-ไอโซไทอะโซลิล ไทโอยูเรีย 4 ตัว

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ลายมือชื่อนิสิต .....
ลายมือชื่ออาจารย์ที่ปรึกษา .....

SUMANAS AKARAPANICHKORN : SYNTHESIS OF ISOTHIAZOLOPYRIMIDINE DERIVERTIVES AS POTENTIAL ANTIMICROBIAL AGENTS. THESIS ADVISOR : ASST. PROF. CHAMNAN PATARAPANICH, Ph.D. AND LECT. SUWANNA VANGVERAWONG, M. Sc. , 114 PP.

This study was to synthesized the new organic compounds which ecpected to have antimicrobial activity and to study the synthetic process of isothiazolopyrimidine derivatives.

The synthesis of isothiazolopyrimidine derivatives, starting from the reaction of 3,5-diaminoisothiazole derivatives and isothiocyanates to yield the N-isothiazolyl thioureas which were then cyclized by heating yielded eight desired products, four 5-substituted-3-substituted aminoisothiazolo[3,4-d]pyrimidine-4-one-6(7H)-thiones and four 5,7-disubstituted-3-aminoisothiazolo[5,4-d]pyrimidine-4-imine-6-thiones; and also eight intermediates, two 3-amino-4-carbethoxy-5-substituted aminoisothiazoles, two 3-amino-4-cyano-5-substituted aminoisothiazoles and four N-substituted-N'-(4-carbethoxy-5-substituted aminoisothiazolyl)-thioureas.

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ลายมือชื่ออาจารย์ที่ปรึกษา .....

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LIST OF ^{1}H NMR SPECTRA

CHAPTER I

INTRODUCTION

The term "antimicrobial agent" may be used to designate some substances of natural, semi-synthetic, or synthetic origin that inhibits or kills free-living, commensal or pathogenic microorganism while causing little or no injury to the host.(1) Some of these are bacteriostatic, the inhibition of growth being reversed when the drug are removed. Others are bactericidal, exerting an irreversible, lethal effect.(2) Antimicrobial agents are employed as antiseptics, disinfectances and preservatives for foods, cosmatics and drugs. Despite the rather large number of known antibiotics, each has important physical, chemical and toxicological limitation so that the interest in the development of new and better antimicrobial remains high.

The many antimicrobial agents available include agents which interfere with cell wall biosynthesis, such as the penicillins, cephalosporins, monolactams and vancomycins. There are also drugs that interfere with protein biosynthesis, such as erythromycin, chloramphenicol and clindamycin, and bactericidal agents such as the aminoglycosides. If there are so many antimicrobial agents, why is it necessary to search a new compounds. The reson

for these are that bacteria are extremely adapt and becoming resistant to the agents.

At present, none of the antimicrobial agents availably possess perfect properties. The future of antimicrobial chemotherapy will be very exciting in which there will be significant progress in the chemotherapy of infection by the use of new agents currently available and those that are in development. It is resonable to seek a new type of chemical that is suitable as and antimicrobial agent.

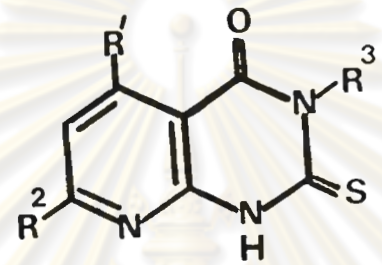
Since the discovery of the antimicrobial activity of sulfonamides in the 1930s, knowledge of the biochemical pathways of cells, particularly of nucleic acid biosynthesis, has been notably expanded. The importance of nucleic acid in protein synthesis, cell divistion, and intracellular synthesis of all essential nucleotides had made chemical modification of the molecular components of nucleic acid a suitable approach for chemotherapy of bacterial infactions as well as of cancer.(3) The hope for discovering new antimicrobial agents could be replaced by the rationally base synthesis of antimetabolite, that might affect nucleic acid and protein biosynthesis. A large number of purine, pyrimidine and amino acid analogs have been synthesized and proved to effective inhibitors of microbial growth.

Some amino acid analogs (e.g., p-fluorophenylalanine) can replace as much as 50% of the corresponding amino acid in the protein being formed, whereas others (e.g., 5-methyltryptophan) inhibit growth without substantially incorporated. The incorporation may or may not impair the function of the product. Thus, when p-fluorophenylalanine replaces much of the phenyl alanine and tyrosine in protein in Escherichia coli, the altered ß-galactosidase formed is still functional but the ß-galactosidase transport protein is not.(2)

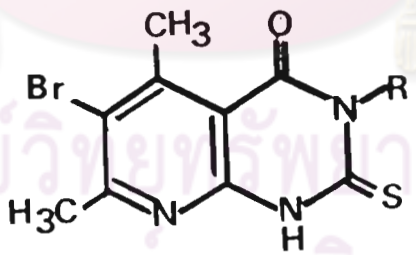
In most case, when purine and pyrimidine analogs inhibit purine metabolism, they usually have to be converted to the corresponding nucleotides lethal form (which constitutes a lethal synthesis) by the enzymes that converted normal base and nucleosides to nucleotides and the enzymes responsible for this kind of conversion are phosphorylase, which usually required the presence of adenosine triphosphate (ATP). Some antimetabolites, however, can act as inhibitors without a kind of activation. (3)

Quinazolone derivatives are known to exhibit a variety of pharmacological properties,(4) 2-alkylthio-pyrido[2,3-d]pyrimidine-4(3H)-ones possessing long lasting diuretic and selective natriuretic activities (5) and some pyridopyrimidinethiones having analgesic antiinflammatory and CNS depressing activities (6-8) are also reported. In addition 2- thiopyrido[2,3-d]pyrimidine-4(3H)-ones (I) and

2-thio-3-substituted-5,7-dimethyl-6-bromopyrido[2,3-d]pyrimidine-4(3H)-ones (II), which are pyridine isosteres of active quinazolones, were synthesized for pharmacological screening. These compound were found to posses significant antimicrobial and antihistaminic activities.(9,10)



I



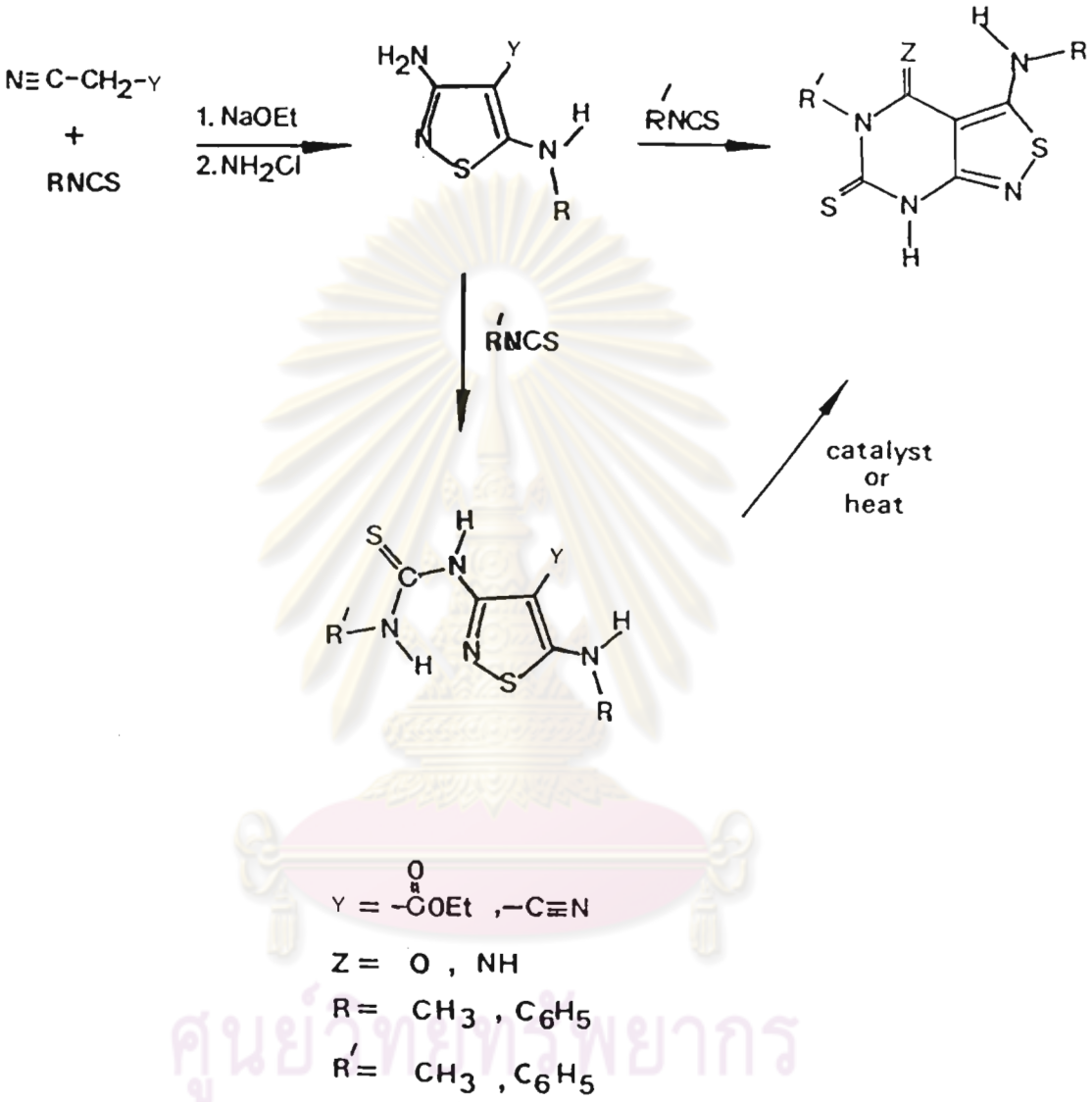
II

This prompted me to synthesize a ring system, isothiazolopyrimidine, which is I and II isostere, in which the -CH=CH- being replaced by a sulfur atom. The electronic

similarly between -S- and -CH=CH- particularly in aromatic systems, has frequently been discussed (e.g., by Longuet-Higgin) (11,12), and isothiazole might, therefore, be expected to resemble pyridine in many of its properties.

It is the purpose of this research study to find a new type of antimicrobial agents. The isothiazolopyrimidines, resulting from cyclization of the coresponding N-isothiazolylthioureas is the target compounds specifically 5-substituted-3-substituted aminoisothiazolo [3,4-d]pyrimidine-4-one-6(7H)-thiones and 5-substituted-3-substituted aminoisothiazolo[3,4-d]-pyrimidine-4-imine-6(7H)-thiones.

The synthetic approach for 5-substituted-3-substituted aminoisothiazolo[3,4-d]pyrimidine-4-one-6(7H)-thiones and 5-subsituted-3-substituted aminoisothiazolo-[3,4-d]pyrimidine-4-imine-6(7H)-thiones can be represented by following schemes:



Scheme 1: Synthesis of 5-substituted-3-substituted amino-isothiazolo[3,4-d]pyrimidine-4-one-6(7H)-thiones and 5-substituted-3-substituted aminoisothiazolo [3,4-d]pyrimidine-4-imine-6(7H)-thiones

CHAPTER II

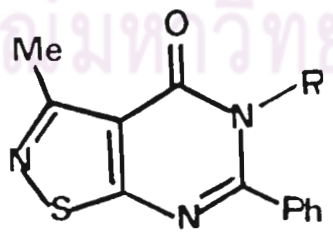
HISTORY

BIOLOGICAL ACTIVITY OF ISOTHIAZOLOPYRIMIDINE DERIVATIVES

Isothiazolopyrimidine derivatives possess numerous important biological properties such as antiviral, sedative, diuretic, antiinflammatory, potential cyclic nucleotide phosphodiesterase inhibitor and nucleoside analog. The important activities are summerized in the following discussion.

1. Antiviral Activity

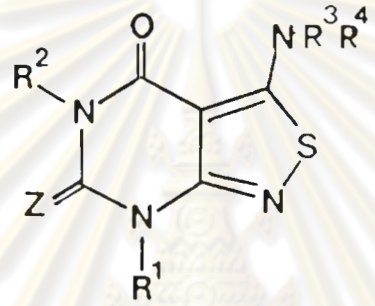
In 1970 , isothiazolopyrimidines (III) has been reported to have potent antiviral activity and show some cytotoxic effect in tumor cell. (13)



III

2. **Antiinflammatory Activity**

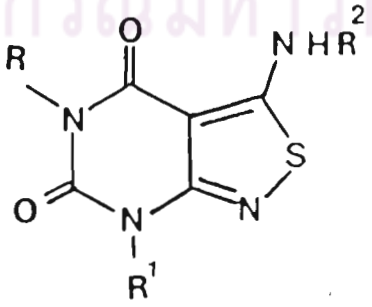
3-Amino isothiazolo[3,4-d]pyrimidine derivatives are reported to have antiinflammatory action. (14) IV has adenosine 3',5'-cyclic phosphate phosphodiesterase inhibiting activity and are useful antiinflammatory agent and sedative.



IV

where R^1 = R^2 = alkyl, aryl ; R^3 = R^4 = alkyl ; Z = O , S

Some 3-amino isothiazolo[3,4-d]pyrimidines(V) have diuretic and antiinflammatory activity, (15) especially when R = R^1 = Pr; R^2 = H.



V

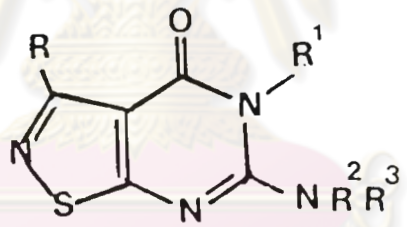
where R = H, Me, Bu, Pr, CH_2Ph

R^1 = Me, Et, Pr, Bu, Ph, CH_2Ph, $(CH_2)_3OMe$, $(CH_2)_3OEt$,

R^2 = H

3. **Sedative Activity**

3-Aminoisothiazolo[3,4-d]pyrimidine derivatives (IV) have sedative activity (14) and 6-amino-4-oxo-5,4-dihydroisothiazolo[5,4-d]pyrimidines (VI) show psychotropic and hypnotic sedative effects. (16)



VI

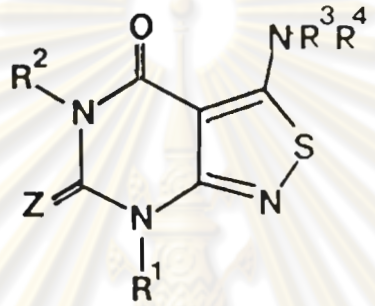
where $R = R^1 = Me$; NR^2R^3 = morpholino

4. **Diuretic Activity**

3-Aminoisothiazolo[3,4-d]pyrimidine (V) having diuretic activity is reported. (15)

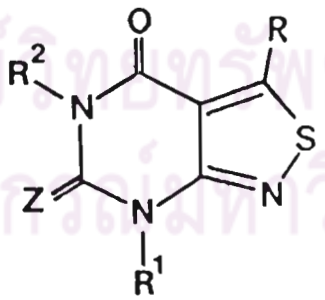
5. Cyclic Nucleotide Phosphodiesterase Inhibitor Activity

In 1976, aminoisothiazolopyrimidines (VII, VIII) have been reported for potential cyclic nucleotide phosphodiesterase inhibitors. (17, 18)



VII

where $R^1 = R^2 = Et$; $R^3 = R^4 = CH_3$; Z = S, O



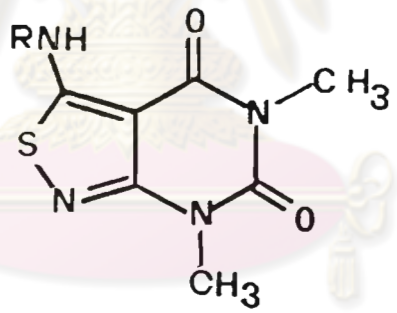
VIII

where $R = NHR^3$, NR^3R^4 ; $R^1 = R^2 = Et$; $R^3R^4 = Me$;

Z = S , O

6 **Nucleoside Analog**

In 1976 and 1978, isothiazolopyrimidine derivatives for instance 3-glycosylamino-5,7-dimethyl isothiazolo[5,4-d]pyrimidine-4,6-diones were synthesized as the nucleotide analogs (19, 20) and in 1980, 1983 disaccharide aminoisothiazolopyrimidine, 5,7-dimethyl, 3[2,3,6-tri-O-acetyl- ∝-D-glucopyranosyl]- ß-D-glucopyranosyl]-ß-D-glucopyranosylaminoisothiazolo[3,4-d]pyrimidine-4,6-diones were synthesized as the nucleoside related compounds. (22, 23)



IX

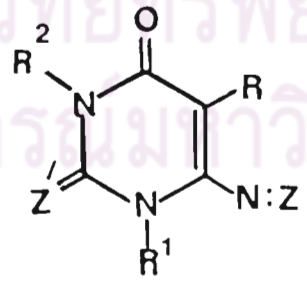
SYNTHESIS OF ISOTHIAZOLOPYRIMIDINE DERIVATIVES

Several synthetic methods of isothiazolopyrimidine derivatives were reported and variations in each method have been applied. Followings are the reported isothiazolo-pyrimidine syntheses

1. Reaction of aminouracil with thionyl chloride
2. Reaction of aminouracil or amino acid with isothiocyanate
3. Reaction of aminouracil with carbon disulfide and methylsulfate, then treat with iodine, follow by nucleophilic substitution by amine

Reaction of aminouracil with thionyl chloride

An one step synthesis of 3-aminoisothiazolo[3,4-d] pyrimidine can be carried out by the reaction of 6-amino-uracil and Vilsmeier's reagent.(17) The aminouracil (X) reacted with dimethylformamide and thionyl chloride yields the dimethylaminoisothiazolopyrimidinedione (XI a) as major product and the uracil (XI b, XII, XIII) as minor products. However, the S analog (XIV), reacted with dimethylformamide and thionyl chloride to give very little of its analog (XV).

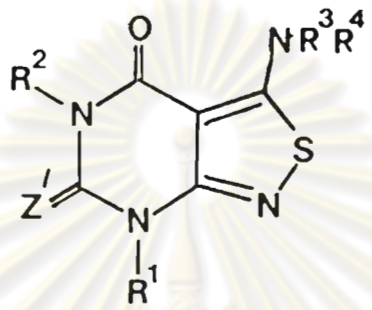


X : R = H ; $R^1 = R^2$ = Et ; Z = H_2; Z′ = O

XII : R = Cl ; $R^1 = R^2$ = Et ; Z = $CHNR^3R^4$; Z′ = O

XIII : R = CHO ; $R^1 = R^2$ = Et ; Z = $CHNR^3R^4$; Z′ = O

XIV : R = H ; $R^1 = R^2$ = Et ; Z = H_2 ; Z′ = S

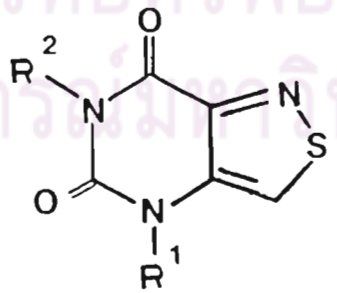


XI a : $R^1 = R^2$ = Et ; $R^3 = R^4$ = Me ; Z′ = O

XI b : $R^1 = R^2$ = Et ; R^3 = Me ; R^4 = H ; Z′ = O

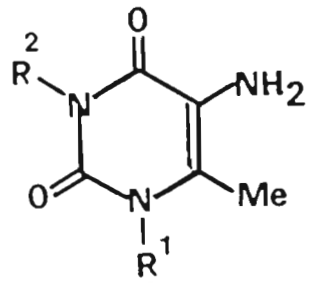
XV : $R^1 = R^2$ = Et ; R^3 = Me ; Z′ = O

Isothiazolo[4,3-d]pyrimidine derivatives (XVI) were prepared with 26-80% yield by treating the aminouracil (XVII) with thionyl chloride. (23)



XVI

where R^1 = Me , Ph ; R^2 = Me , Ph , Me_2



XVII

where R^1 = Me , Ph ; R^2 = Me , Ph , Me_2

Reaction of aminouracil or amino acid with isothiocyanate

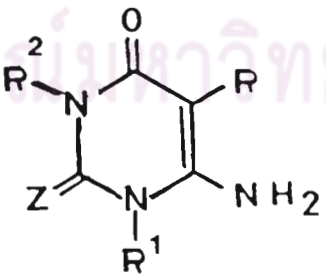
The isothiazolo[3,4-d]pyrimidine 4,6-(5H, 7H)dione derivatives (XVIII), were prepared by oxidative cyclization of the carboxamides (XIX b) which were prepared by reaction of (XIX a) with corresponding isothiocyanates. (24)

XVIII : R^1 = H or Pr ; R^2 = Me or Bu ; R^3 = H , $SO_2C_6H_4Me$-4 or CO_2Et

XIX a : R^1 = H or Pr ; R^2 = Me or Bu ; R^4 = $CSNHR^3$

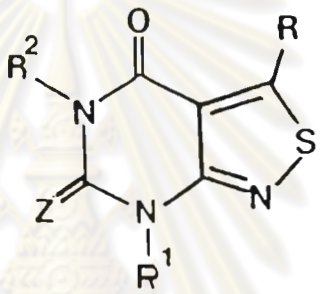
XIX b : R^1 = H or Pr ; R^2 = Me or Bu ; R^4 = H

The 6-aminouracil(XX) reacted with isothiocyanates (RNCS, R^3 = Et, Me, C_6H_4Cl-p, Ph, C_6H_4OH-p, CH_2Ph) to give thiocarbamoyluracils (XXI), which were oxidized with bromine or hydrogen peroxide to give the isothiazolopyrimidinedione (XXII), whose alkylation with (XX) gave the disubstituted amino isothiazolopyrimidines (XXIII). (18)



XX : R = H ; R^1 = $PhCH_2$, Et , H , Me , p-ClC_6H_4 ,
$MeOCH_2CH_2$, Ph , Me CH_2CH_2 , p-$MeOC_6H_4$;
R^2 = H , Et ; Z = O , S

XXI : R = CSNHR ; R^1 = $PhCH_2$, Et , H , p-ClC_6H_4 ,
$MeOCH_2CH_2$, Ph , $MeCH_2CH_2$, p-$MeOC_6H_4$;
R^2 = H , Et; Z = O , S

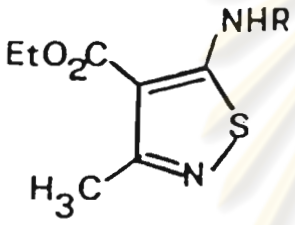


XXII : R = NHR^3 ; R^1 = $PhCH_2$, Et , H, Me ,
p-ClC_6H_4, $MeOCH_2CH_2$, Ph , $MeCH_2CH_2$,
p-$MeOC_6H_4$; R^2 = H , Et ; Z = O , S

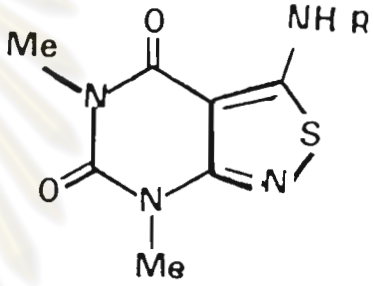
XXIII : R = NR^3R^4 ; R^1 = $PhCH_2$, Et , H , Me ,
p-ClC_6H_4 ; $MeOCH_2CH_2$, Ph , $MeCH_2CH_2$,
p-$MeOC_6H_4$; R^2 = H , Et ; Z = O , S

Isothiazolopyrimidine derivatives can also be obtained the reaction of enamine with D-glucosyl and D-gluconyl isothiocyanate. Treatment of isothiocyanate (RNCS, R = tetra-O-acetyl-β-D-glucopyranosyl, tri-O-acetyl-D-arabinopyranosyl, tri-O-benzolyl-β-D-ribofuranosyl) with $H_2NCMe:CHCO_2Et$ in tetrahydrofuran at room temperature gave

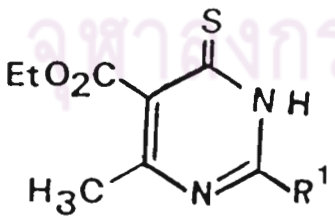
isothiazole (XXIV) and $H_2NCMe{:}C(CO_2Et)C(S)NHR$, Cyclization of isothiocyanate with 6-amino-1,3-dimethyluracil gave isothiazolopyrimidine (XXV). Treatment of penta-O-acetyl-D-gluconyl isothiocyanate with $H_2NCMe{:}CH_2CO_2Et$ and 6-amino-1,3-dimethyluracil gave (XXVI) and (XXVII) (R^1 = penta-O-acetyl-D-gluconyl). (25)



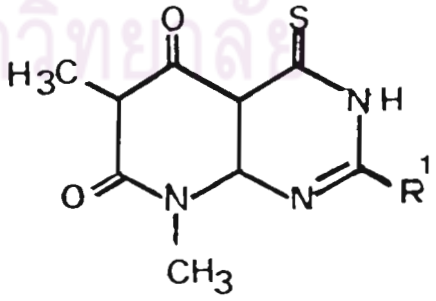
XXIV



XXV



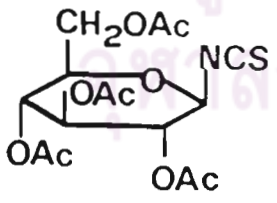
XXVI



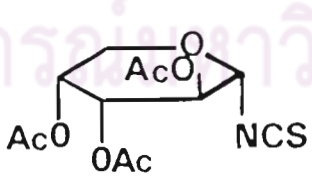
XXVII

3-Aminoglucosyl-5,7-dimethylisothiazolo[3,4-d]pyrimidine-4,6(5H, 7H)-dione can be synthesized by the reaction of glycosyl isothiocyanate by the reaction of glycosyl and gluconyl isothiocynate with amino acids, enamine and diamine. (20)

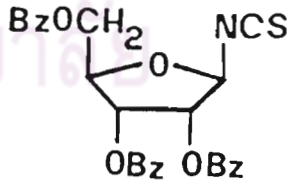
An one step synthesis of glycosylaminoisothiazolo-[3,4-d]pyrimidines were carried out by the reaction of glycosyl isothiocyanate (XXVIII) with 2-amino pyridine or 2-amino-4-picoline to give N-glycosyl-N′-(2-pyridyl)-thiourea and N-glycosyl-N′-(4-methyl-2-pyridyl)-thiourea, respectively, in good yields, but the cyclized products were not obtained. On the other hand the reaction of glycosyl isothiocyanate (XXVIII), (XXIX), and (XXX) with $MeC(NH_2):CHCO_2Et$ gave $MeC(NH_2):C(CSglycosyl)$. Similar reaction of (XXVIII)-(XXX) with 6-amino-1,7-dimethyluracil gave nucleotide analogs(XXXII). (21)



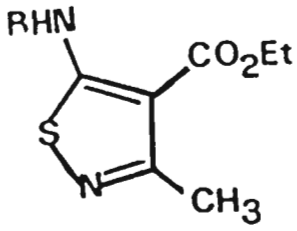
XXVIII



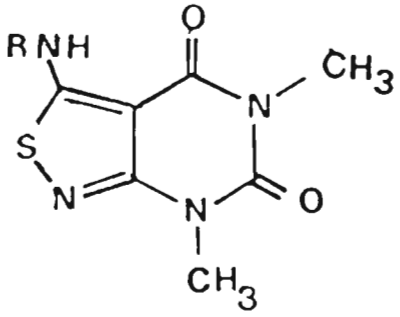
XXIX



XXX

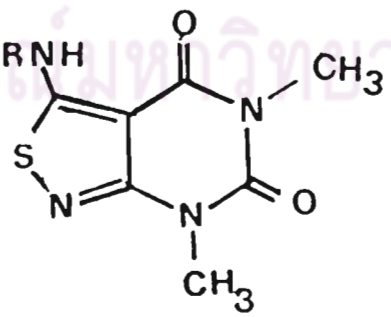


XXXI



XXXII

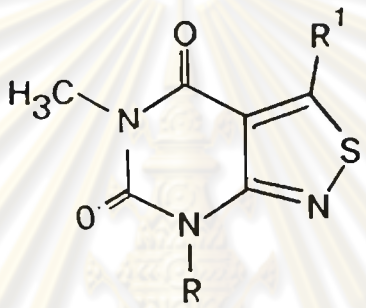
In 1983, modified nucleotide analogs 5,7-dimethyl-3[2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-∝-D-glucopyranosyl)-ß-D-glucopyranosylamino]-isothiazolo[3,4-d]-pyrimidine-4,6-dione, were prepared starting from hepta-O-acetyl-ß-lactosyl isothicyanate, hepta-O-acetyl-ß-meltosyl isothiocyanate. Reaction of hepta-O-acetyl-ß-lactosyl isothiocyanate, hepta-O-acetyl-ß-meltosyl isothiocyanate with 6-amino-1,3-dimethyluracil gave disaccharide aminoisothia-zolopyrimidines (XXXIII) in good yields. (19)



XXXIII

Reaction of aminouracil with carbondisulfide and methyl sulfate, then treat with iodine follow by nucleophilic substitution by amine

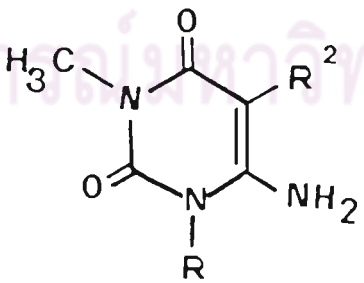
Isothiazolopyrimidinediones (XXXIV) were obtained by treating uracil (XXXV) with iodine (XXXIV) (R^1 = SMe) underwent nucleophilic substitution to give XXXV. (26)



XXXIV : R = Me , Ph ; R^1 = SMe

XXXV : R = Me , Ph ; R^1 = amino , $CH(CN)CO_2Me$

$CH(CN)SO_2Ph$



XXXVI : R = Me, Ph ; R^1 = SMe ; R^2 = CS , Me

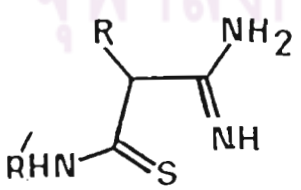
From the synthetic methods above, it can be concluded that isothiazolopyrimidines were obtained by the reaction starting from aminouracil. According to the synthetic planning (scheme I), isothiazolopyrimidines can be synthesized in 3 steps:

1. Synthesis of 3,5-diaminoisothiazoles
2. Synthesis of N-isothiazolyl thioureas
3. Cyclization of N-isothiazolyl thioureas

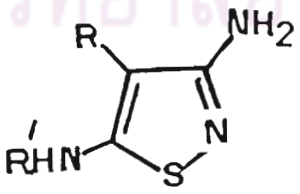
Step 1: SYNTHESIS OF 3,5-DIAMINOISOTHIAZOLES

The synthesis of 3,5-diaminoisothiazoles can be carried out by oxidative cyclization or aminative cyclization of appropriate open chain substrates.

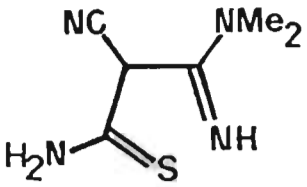
3,5-Diaminoisothiazoles (XXXVIII and XL) can be carried out by the oxidative cyclization of the corresponding thiocarbamoyl acetronitrile (XXXVII) and thiocarbamoyl amidines (XXXIX). (27)

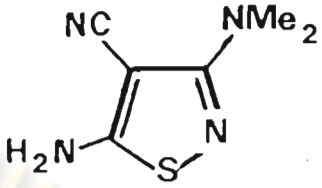


XXXVII



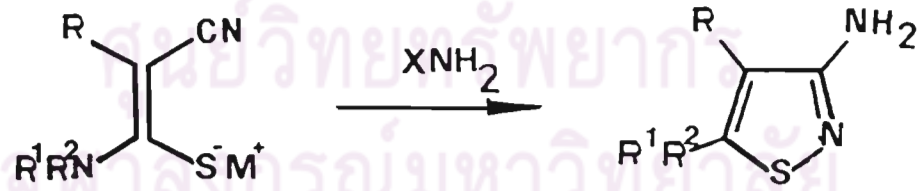
XXXVIII





XXXIX XL

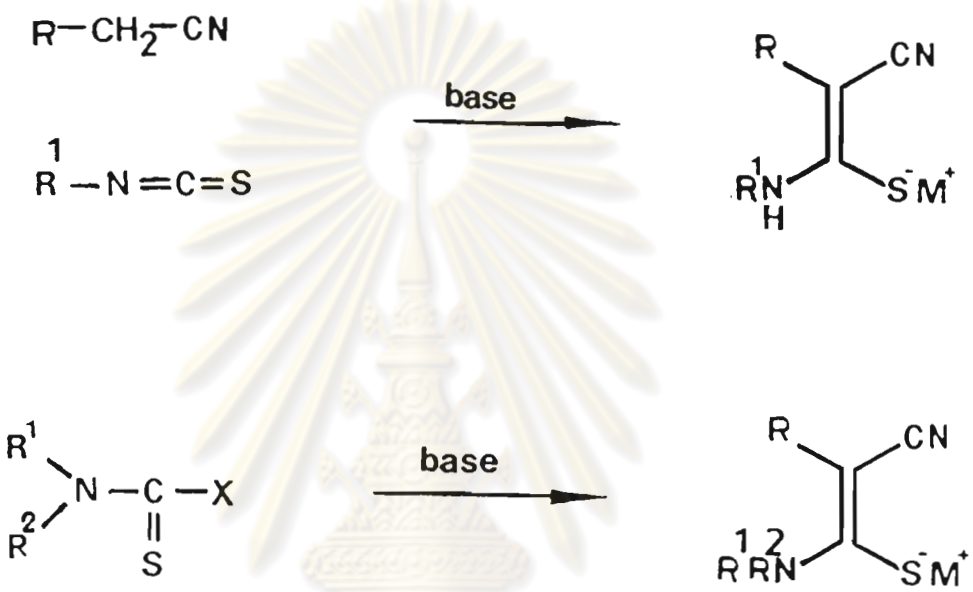
The aminative cyclization of 3-amino-3-mercapto-acrylonitriles to yield 3,5-diaminoisothiazoles was also reported. (27)



X = halogen or other leaving group

M^+ = alkali metal

The 3-amino-3-mercaptoacrylonitriles were synthesized by the reaction of active methylene nitriles with isothiocyanates or other thiocarbamoylating agents.(28, 29)



M^{+} = H^{+}, alkali metal or ammonium ion

X = leaving group

Step 2: SYNTHESIS OF N-ISOTHIAZOLYL THIOUREAS

N-Isothiazolyl thioureas were synthesized by the general synthesis of thiourea derivatives. Generally, there are several commom methods for synthesizing thiourea derivatives, and variations in each methods have been applied. Following are the synthetic method of thioureas.

1. Reaction of carbondisulfide and an amine.

This method is the most common method for synthesizing 1,3-disubstituted thiourea having the same substitutents. 1,3-Disubstituted thioureas with dissimilar substitutents can not be prepared by this method. Since this reaction is often slow, certain compounds such as sulfur (30), hydrogen peroxide (31), potassium or sodium hydroxide (32), iodine and pyridine (33) have been successfully employed to accelerate the reaction.

2. Reaction of thiophosgene and an amine.

The product of this reaction can be the isothiocyanate or the 1,3-disubstituted thiourea, depending on the ratio of thiophosgene and the amine used.

Preparation of thioureas by this method is best carried out by refluxing one mole of thiophosgene and two moles of the amine in aqueous (34), aqueous chloroform(35), or aqueous acetone solution (36). when thiophosgene has completely reacted, a mole of potassium carbonate is added, the heating is continued for several hours; finally, the desired product can be obtained.

Because of the nature of thiophosgene, this method is usually reserved for the instances when other methods fail to give the desired product.

3. Reaction of organic isothiocyanate and an amine

This is the best method for preparing unsymmetrical substituted thioureas. Ammonia, primary amines and secondary amines can be used whereas R, R′ and R″ may be aromatic, aliphatic, alicyclic or heterocyclic amines. The addition of amine to isothiocyanate is usually carried out in the presence of a solvent like alcohol, benzene, toluene, acetone, chloroform, pyridine or ether.(37) 1-Mono, 1,1- or 1,3-disubstituted or 1,1,3-trisubstituted thioureas can be synthesized by this method with good yields. The reaction is also widely used for characterizing amine compounds.

4. Reaction of alkaline thiocyanate and amine hydrochloride.

Heating ammonium thiocyanate at 160 °C. for several hours causes it to rearrange to thiourea. (38)

The reaction can be carried out in either an inert organic solvent (39), or in aqueous medium (40), and 1-mono substituted or 1,1-disubstituted thioureas can be obtained but not 1,3-disubstituted thioureas.

5. Miscellaneous methods

Thiourea react with acyl, alkyl, arakyl and heterocyclic halides to give thiourea derivatives. Generally, S-acylation occurs first and upon heating, or sometime merely upon standing at room temperature, the acyl group transfer to nitrogen. (41) Alkyl, arakyl and heterocyclic halides give stable S-substituted products with thioureas and this is the most method of preparing pseudo thioureas. The reaction may be carried out by mixing

a 1 : 1 molar ratio of the reactants directly in an innert solvent (42) or in anhydrous ethanol. (43)

Step 3: CYCLIZATION OF N-ISOTHIAZOLYL THIOUREAS

The cyclization of straight chain substrates which have nucleophilic group and position to the carbonyl group or cycno group can be brought about by gentle heating, in the presence of mild dehydrating agents suchs as acid acid catalyst or base catalyst to form five or six-membered rings. (44, 45, 46,)

Many organic reactions are initiated by a first step involving either protonation or deprotonation of reactant which is a base or an acid, respectively. This is done by applying an acid or base as reagent. This initiation acid or base catalysts should be chosen so as sufficient acidic strength be obtained. This is assesed by comparing the reagent and reactant pKa's. If a reagent is chosen that causes the equilibrium to favour the starting materials and hence yield only a small proportion of products, the choice will be acceptable only if this small equilibrium supply does not choke off the subsequence desired reaction of those products. (47, 48)

Reagent base must also be chosen so as not to attack other positive sites than acidic protons in the molecule or else side reactions will occur. The common reagent used are sodium hydride, tertiary butyl lithium,

lithium diethylamine, sodium amine, potassium tertiary butoxide, sodium methoxide, sodium hydroxide, triethyl amine, sodium acetate and pyridine. The base sodium hydride and tertiary butoxide are commonly used for selective attack on proton rather than other positive sites. The last three base due to their steric hindrance, prohibits attack on sites bulkier than protons. Acids, fluosulfonic acid, fluoboric acid, sulfuric acid, boron trifluoride, benzene sulfonic acid, hydrochloric acid, trifluoroacetic acid and acetic acid , must sometimes be chosen so that they offer no mild base counter ion which is capable to attack the protonated reactant; fluorosulfonic acid and boron trifluoride are common for this purpose. Lewis acid and charge transfer complexes are extentions of the acid-base reaction beyond protons (acids) and unshared pairs (base) to include as well electron deficient species as acid and electron as bases. Acid base reactions are important both as initiator if subsequent reactions of many kinds and a model for more complex reactions. (48)

CHAPTER III

EXPERIMENTAL

Elemental Analysis

Analysis for carbon, hydrogen, nitrogen and sulfur were performed on a Perkin Elmer 240 C Elemental Analyser by the laboratory of Scientific and Technological Research Equipment Center, Chulalongkorn University, Bangkok.

Melting Point

All melting points were taken on Buchi capillary melting point apparatus and are uncorrected.

Infrared Absorption Spectra

All infrared absorption spectra were obtained with a IR-440 Infrared Spectrophotometer by the laboratory of Scientific and Technological Research Equipment Center, Chulalongkorn University, Bangkok.

Nuclear Magnetic Resonance Spectra

All nuclear magnetic resonance spectra were obtained from a FX-900 Nuclear Magnetic Resonance Spectrophotometer by the laboratory of Scientific and

Technological Research Equipment Center, Chulalongkorn University, Bangkok.

Chemical

The starting materials used are, ethyl cyanoacetate (BDH chemicals Ltd.), malononitrile (Sigma Chemical Co.), methyl isothiocyanate and phenyl isothiocyanate (Fluka AG,CH-9470 Buchs). All Solvents used were B.P. grade and Laboratory grade.

3-Amino-4-carbethoxy-5-methylaminoisothiazole

To a solution of sodium ethoxide, prepared by dissolving 0.6 gm (0.025 mole) sodium metal in 25 ml of absolute ethanol, was added 2.83 gm (0.025 mole) of ethyl cyanoacetate, followed by 1.851 gm (0.025 mole) of methyl isothiocyanate. The mixture was refluxed for 10 minutes, and allowed to stired at room temperature for 12 hours. The solution was added to cold aqueous chloramine solution, which prepared from 50 ml of 5% sodium hypochlorite solution in 100 gm ice and 10 ml of 25% ammonium hydroxide. The reaction mixture was stirred at room temperature for 12 hours and the solid obtained was filtered, washed and dried. Recrystallisation from ethanol yielded 2.5 gm (50.0% yield) crystals, mp 153-155 ^{o}C. (27)

IR (KBr): 3500, 3380 (NH_2); 3300 (NH); 3000 (=CH, CH); 1680 (C=O); 1240 (C-O) $cm.^{-1}$ (figure 1)

^{1}H-NMR ($CDCl_3$): 1.37 (t, J = 7, 3H, $-CH_2-CH_3$); 2.93 (d, J = 5.4, 3H, $-NH-CH_3$) ; 4.35 (q, J = 7 , 2H , $-O-CH_2-CH_3$); 5.48 (s, 2H, isothiazole-NH_2) ; 7.79 (br, 1H, $-NH-CH_3$). (figure 19)

3-Amino-4-carbethoxy-5-phenylaminoisothiazole

To a solution of sodium ethoxide, prepared by dissolving 0.6 gm (0.025 mole) of sodium metal in 25 ml absolute ethanol, was added 2.83 gm (0.025 mole) of ethyl cyanoacetate, followed by 3.38 gm (0.025 mole) of phenyl isothiocyanate. The mixture was sitrred over night, then the solution was poured into cold aqueous chloramine solution, prepared by mixing 50 ml of 5% sodium hypochlorite, 10 ml of 25% ammonium hydroxide and 100 gm ice. The mixture was stirred overnight and offwhite precipitate formed. The product was collected by filtration, washed and dried. The total product was 5.88 gm (89.5 % yield). Recrystallisation from ethanol gave crystals; mp 159-160 ^{o}C. (27)

IR (KBr): 3500, 3300 (NH_2); 3180 (NH); 3000 (=CH, CH); 1660 (C=O); 1220(C-O) cm^{-1}. (figure 2)

^{1}H-NMR ($CDCl_3$): 1.43 (t, J = 7, 3H, $-CH_2-CH_3$); 4.37(q,J = 7,2H,$-O-CH_2-CH_3$);5.48 (s, 2H, isothiazole-NH_2); 7.18 (m, 5H, $-NH-C_6H_5$); 9.97 (br, 1H, $-NH-C_6H_5$). (figure 20)

3-Amino-4-cyano-5-methylaminoisothiazole

To a solution of sodium ethoxide, prepared by dissolving 0.6 gm sodium metal in 25 ml of absolute ethanol, was added 1.65 gm (0.025 mole) of malononitrile, followed by 1.85 gm (0.025 mole) of methyl isothiocyanate. The mixture was refluxed for 10 minutes, and allowed to stir at room temperature for 12 hours. The solution was added to cold aqeous chloramine solution, prepared from 50 ml of 5% sodium hypochlorite solution in 100 gm ice and 10 ml of 25% ammonium hydroxide. The reaction mixture was stirred at room temperature for 12 hours and the solid obtained was filtered, washed and dried. Recrystallisation from ethanol yielded 2.31 gm (60.0% yield) crystals, mp 255-256 ^{o}C.

IR (KBr): 3450, 3360 (NH_2); 3200 (NH); 3000 (=CH, CH); 2200 (C≡N); 1625 (C=N); 1400 (CH_3) cm^{-1}. (figure 3)

^{1}H-NMR ($CDCl_3$,d_6-DMSO): 3.80 (d, J = 6, 3H, CH_3-NH-); 5.96 (s, 2H, isothiazole-NH_2); 8.09 (m, J = 6, 1H, -NH-CH_3). (figure 21)

Analysis for $C_5H_6N_4S$:

Calcd.	C = 38.95	H = 3.92	N = 36.33
Found	C = 38.70	H = 3.85	N = 36.29

3-Amino-4-cyano-5-phenylaminoisothiazole

To a solution of sodium ethoxide, prepared by dissolving 0.6 gm (0.025 mole) of sodium metal in 25 ml absolute ethanol, was added 1.65 gm (0.025 mole) of malononitrile, followed by 3.38 gm (0.025 mole) of phenyl isothiocyanate. The mixture was stirred overnight, then the solution was poured into cold aqueous chloramine solution, prepared by mixing 50 ml of sodium hypochlorite, 10 ml of 25% ammonium hydroxide and 100 gm of ice. The mixture was stirred overnight and offwhite precipitate formed. The product was collected by filtration, washed and dried. The total product was 3.24 gm (40.0% yield). Recrystallisation from ethanol gave crystals; mp 230-231 °C.

IR (KBr): 3450, 3340 (NH_2); 3200 (NH); 2200 (C≡N); 1630 (C=N) cm^{-1}. (figure 4)

^{1}H-NMR ($CDCl_3$, d_6-DMSO): 5.81 (s, 2H, isothiazole-NH_2); 7.29 (m, 5H, -NH-C_6H_5); 10.12 (b, 1H, -NH-C_6H_5). (figure 22)

Analysis for $C_{10}H_8N_4S$:

Calcd. C = 55.54 H = 3.72 N = 25.91

Found C = 55.42 H = 3.69 N = 25.90

N-Methyl-N -(4-carbethoxy-5-methylamino-3-isothiazolyl)-thiourea

To a solution of 0.4025 gm (0.002 mole) 3-amino-4-carbethoxy-5-methylaminoisothiazole in 10 ml pyridine, 0.1222 gm (0.002 mole) methyl isothiocyanate was added.

The solution was refluxed for 3 hours. After cooling, the reaction the mixture was poured onto crushed ice, then separated solid was collected, washed with water. The collected product was dried and finally, recrystallised with ethanol to give silky needle crystals which were then dried in a desiccator over anhydrous $CaCl_2$. The total yield was 0.0988 gm (18.0% yield), mp 133-135 °C.

IR (KBr): 3400 (NH); 3280 (NH); 3200 (NH); 3000 (=CH, CH); 1720 (C=O); 1400 (CH_3); 1380 (CH_3); 1210 (C=S); 1200 (C-O) cm^{-1}. (figure 5)

^{1}H-NMR ($CDCl_3$): 1.45 (t, J = 7, 3H, CH_3-CH_2-); 3.00 (d, 3H, CH_3-NH-); 3.20 (d, 3H, CH_3-NH-); 4.44 (q, J = 7, 2H, CH_3-CH_2-O-); 7.40 (b, 1H, -NH-CH_3); 10.20 (b, 1H, -NH-); 10.57 (b, 1H, -C(S)-NH-CH_3). (figure 23)

Analysis for $C_9H_{14}N_4O_2S_2$:

Calcd. C = 39.40 H = 5.14 N = 20.42

Found C = 39.36 H = 5.20 N = 20.26

N-Phenyl-N'-(4-carbethoxy-5-methylamino-3-isothiazolyl)-thiourea

After added 0.2268 gm (0.002 mole) phenyl isothiocyanate to a solution of 0.4025 gm (0.002 mole) 3-amino-4-carbethoxy-5-methylaminoisothiazole in 10 ml pyridine, the solution was refluxed for 3 hours. The reaction was stopped by cooling, the mixture was poured onto crushed ice, then the solid separated was collected, washed with water , dried and finally recrystallised with

ethanol to give silky needle crystals. The total yield was 0.3701 gm (55.0% yield), mp 169-171 °C.

IR (KBr): 3400 (NH); 3280 (NH); 3000 (=CH, CH); 1685 (C=O); 1650-1450 (aromatic); 1390 (CH_3); 1220 (C=S); 775, 740 (monosubstituted aromatic) cm^{-1}. (figure 6)

^{1}H-NMR ($CDCl_3$): 1.46 (t, J = 7, 3H, $-CH_2-CH_3$); 3.00 (d, J = 6, 3H, $-NH-CH_3$); 4.40 (q, J = 7, 2H, $-O-CH_2-CH_3$); 7.40 (m, 6H, $-NH-C_6H_5$ and $-NH-CH_3$), 10.24 (s, 1H, -NH-); 12.49(br, 1H, $-C(S)-NH-C_6H_5$). (figure 24)

Analysis for $C_{14}H_{16}N_4O_2S_2$:

Calcd.	C = 49.98	H = 4.79	N = 16.65
Found	C = 49.91	H = 4.79	N = 16.51

N-Methyl-N'-(4-carbethoxy-5-phenylamino-3-isothiazolyl)-thiourea

To a solution of 0.5266 gm (0.002 mole) 3-amino-4-carbethoxy-5-phenylaminoisothiazole in 10 ml pyridine, 0.1222 gm (0.002 mole) methyl isothiocyanate was added. The solution was refluxed for 3 hours. After cooling the reaction, the mixture was poured onto crushed ice, separated solid , collected, washed with water, dried and finally recrystallised with ethanol to give silky needle crystals, which were then dried in a desiccator over anhydrous $CaCl_2$. The total yield was 0.2153 gm (32.0% yield), mp 148-150 °C.

IR (KBr): 3400 (NH); 3240 (NH); 2980 (=CH, CH); 1664 (C=O); 1620-1450 (aromatic); 1375 (CH_3); 1250 (C=S); 1220 (C-O); 775, 740 (monosubstituted aromatic) cm^{-1}. (figure 7)

H-NMR ($CDCl_3$): 1.50 (t, J = 7, 3H, $-CH_2-CH_3$); 3.22 (d, J = 6 , 3H, $-NH-CH_3$); 4.55 (q, J = 7, 2H, $-O-CH_2-CH_3$); 7.20 (m, 5H, C_6H_5-NH-); 10.00 (b, 2H, -NH-, $-NH-C_6H_5$); 10.48 (b, 1H, $-C(S)-NH-CH_3$). (figure 25)

Analysis for $C_{14}H_{16}N_4O_2S_2$:

Calcd.	C = 49.98	H = 4.79	N = 16.65
Found	C = 50.32	H = 4.78	N = 16.44

N-Phenyl-N'-(4-carbethoxy-5-phenylamino-3-isothiazolyl)-thiourea

After added 0.2268 gm (0.002 mole) phenyl isothiocyanate to a solution of 0.5216 gm (0.002 mole) 3-amino-4-carbethoxy-5-phenylaminoisothiazole in 10 ml pyridine, the solution was refluxed for 3 hours. The reaction was stopped by cooling, the mixture was poured onto the crushed ice, then the solid was separated, collected, washed with water, dried and finally recrystallised with ethanol to give silky needle crystals. The total yield was 0.5739 gm (72.0% yield), mp 131-132 °C.

IR (KBr): 3350 (NH); 3000 (=CH, CH); 1675 (C=O); 1650-1450 (aromatic); 1225 (C=S)cm^{-1}. (figure 8)

^{1}H-NMR($CDCl_3$): 1.54 (t, J = 7, 3H, $-CH_2-CH_3$); 4.50 (q, J =7, 2H, $-O-CH_2-CH_3$); 7.40 (m, 10H,2 $-NH-C_6H_5$); 10.13 (br, 2H, -NH-, $-NH-C_6H_5$); 12.40 (br, 1H, $-NH-C_6H_5$). (figure 26)

Analysis for $C_{19}H_{18}N_4O_2S_2$:

Calcd. C = 57.26 H = 4.55 N = 14.06

Found C = 57.19 H = 4.55 N = 14.00

5-Methyl-3-methylamino isothiazolo[3, 4-d]pyrimidine-4-one-6(7H)-thione

To a solution of 0.4025 gm (0.002 mole) 3-amino-4-carbethoxy-5-methyl aminoisothiazole in 10 ml pyridine, 0.1222 gm (0.002 mole) methyl isothiocyanate was added. The solution was refluxed for 6 hours. After cooling the reaction the mixture was poured onto crushed ice, separated the solid, collected, washed with water, dried, added ethanol and separated the solid obtained from boiling ethanol, finally recrystallised with dilute sodium hydroxide solution and then dried in a desiccator over anhydrous $CaCl_2$. The total yield was 0.2268 gm (50% yield), mp over 260 ^{o}C.

IR (KBr): 3280 (NH); 3200 (NH); 3000 (=CH, CH); 1660 (C=O); 1398, 1360 (CH_3); 1250 (C=S) cm^{-1}. (figure 9)

^{1}HNMR ($CDCl_3$, d_5-pyridine): (figure 27)

Analysis for $C_7H_8N_4OS_2$:

Calcd. C = 36.82 H = 3.51 N = 24.55

Found C = 36.89 H = 3.56 N = 24.09

5-Phenyl-3-methylamino isothiazolo[3, 4-d]pyrimidine-4-one-6(7H)-thione

To a solution of 0.5261 gm (0.002 mole) 3-amino-4-carbethoxy-5-methylisothiazole in 10 ml pyridine, 0.2268 gm phenyl isothiocyanate was added. The solution was refluxed for 6 hours. After cooling the reaction, the mixture was poured onto crushed ice, separated the solid obtained from boiling ethanol, finally recrystallised with dilute sodium hydroxide solution and then dried in a desiccator over anhydrous $CaCl_2$. The total yield was 0.1858 gm (32 % yield), mp over 260 ^{o}C.

IR (KBr): 3400 (NH); 3330 (NH); 3000 (= CH, CH); 1660 (C=O); 1650-1450 (aromatic) ; 1360 (CH_3); 1200 (C=S); 770,740 (monosubstituted aromatic) cm^{-1}. (figure 10)

^{1}H-NMR ($CDCl_3$, d_5-pyridine) : (figure 28)

Analysis for $C_{12}H_{10}N_4OS_2$:

Calcd. C = 49.63 H = 3.45 N = 19.30

Found C = 49.29 H = 3.33 N = 18.84

5-Methyl-3-phenylamino isothiazolo[3, 4-d]pyrimidine-4-one-6(7H)-thione

To solution of 0.5261 gm (0.002 mole) 3-amino-4-carbethoxy-5-phenylisothiazole in 10 ml pyridine, 0.1222 gm (0.002 mole) methyl isothiocyanate was added. The solution was refluxed for 6 hours. After cooling the reaction, the mixture was poured onto crushed ice, separated solid, collected, washed with water, dried, added ethanol and separated the solid obtained from boiling ethanol, finally recrystallised with dilute sodium hydroxide solution and dried in a desiccator over anhydrous $CaCl_2$. The total yield was 0.2671 gm (46.0 % yield), mp over 260 °C.

IR (KBr): 3400 (NH); 3200 (NH); 1640 (C=O); 1620-1450 (aromatic); 1240 (C=S) cm^{-1}. (figure 11)

^{1}H-NMR ($CDCl_3$, d_5-pyridine) : (figure 29); ($CDCl_3$, d_6-DMSO) : (figure 29)

Analysis for $C_{12}H_{10}N_4OS_2$:

Calcd. C = 49.63 H = 3.45 N = 19.30

Found C = 49.32 H = 3.38 N = 18.84

5-Phenyl-3-phenylamino isothiazolo[3,4-d]pyrimidine-4-one-6(7H)-thione

Phenyl isothiocyanate 0.2268 gm (0.002 mole) was added to a solution of 0.5261 gm (0.002 mole) 3-amino-4-carbethoxy-5-phenylaminoisothiazole in 10 ml of pyridine. The solution was refluxed for 6 hours. After cooling the reaction, the mixture was poured onto the crushed ice,

separated the solid, collected, washed with water, dried, added ethanol and separated the solid obtained from boiling ethanol, finally recrystallised with dilute sodium hydroxide solution and dried in desiccator over anhydrous $CaCl_2$. The total yield was 0.2115 gm (30.0 5 yield) , mp over 260 ^{o}C.

IR (KBr): 3400 (NH); 3300 (NH); 3000 (=CH, CH); 1668 (C=O); 1650-1450 (aromatic); 1200 (C=S); 775, 740 (monosubstituted aromatic $cm.^{-1}$ (figure 12)

^{1}H-NMR ($CDCl_3$, d_5-pyridine): (figure 31)

Analysis for $C_{17}H_{12}N_4OS_2$:

Calcd. C = 57.93 H = 3.41 N = 15.90

Found C = 57.63 H = 3.31 N = 15.79

Attempt to synthesize 5-Methyl-3-methylaminoisothiazolo-[3,4-d]pyrimidine-4-imine-6(7H)-thione

After added 0.1222 gm (0.002 mole) methyl isothiacyanate to a solution of 0.3084 gm (0.002 mole) 3-amino-4-cyano-5-methylaminoisothiazole in 10 ml pyridine, the solution was refluxed for 3 hours, distilled pyridine out, then the solid separated was collected, washed with acetone, dried and finally recrystallised with pyridine. However the product obtained was suspected to be 5,7-dimethyl - 3 -aminoisothiazolo[5,4-d]pyrimidine-4-imine- 6-thione. The total yield was 0.2273 gm (50.0% yield), mp over 260 ^{o}C.

IR (KBr): 3450, 3350 (NH_2); 3250 (NH); 3000 (=CH, CH); 1600 (C=N); 1395 (CH_3); 1250 (C=S) cm^{-1}. (figure 13)

^{1}H-NMR ($CDCl_3$, d_5-pyridine) : (figure 32)

Analysis for $C_7H_9N_5S_2$:

Calcd. C = 36.99 H = 3.99 N = 30.81

Found C = 36.97 H = 3.79 N = 30.65

Attempt to synthesize 5-Phenyl-3-methylaminoisothiazolo-[3,4-d]pyrimidine-4-imine-6(7H)-thione

To a solution of 0.3084 gm (0.002 mole) 3-amino-4-cyano-5-methylaminoisothiazole in 10 ml pyridine, 0.2268 gm (0.002 mole) of phenyl isothiocyanate was added. The solution was refluxed for 3 hours. After pyridinewas distilled, the separated solid was collected and washed with acetone. The collected product was dried and finally, recrystallised with pyridine. However the obtained product was suspected to be 5-phenyl-7-methyl-3-aminoisothiazolo-[5,4-d]pyrimidine-4-imine-6-thione. The total yield was 0.3762 gm (65.0%) , mp over 260 ^{o}C.

IR (KBr): 3400, 3280 (NH_2); 3000 (=CH, CH); 1640 (C=N); 1380 (CH_3); 1250 (C=S); 775, 740 (monosubstituted aromatic) cm^{-1}. (figure 14)

^{1}H-NMR ($CDCl_3$, d_5-pyridine): (figure 33)

Analysis for $C_{12}H_{11}N_5S_2$:

Calcd. C = 49.81 H = 3.83 N = 24.20

Found C = 49.84 H = 3.79 N = 23.08

Attempt to synthesize 5-Methyl-3-phenylaminoisothiazolo-[3,4-d]pyrimidine-4-imine-6(7H)-thione

After adding 0.1222 gm (0.002 mole) of methyl isothiocyanate to a solution of 0.4325 gm (0.002 mole) of 3-amino-4-cyano-5-phenylaminoisothiazole in 10 ml pyridine, the solution was refluxed for 3 hours, distilled pyridine out, then the solid separated was collected, washed with acetone, dried and finally recrystallised with pyridine. However the product obtained was suspected to be 5-methyl-7-phenyl- 3 - aminoisothiazolo[5,4-d]pyrimidine - 4 -imine-6-thione. The total yield was 0.3762 gm (65.0%), mp over 260 ^{o}C.

IR (KBr): 3450 (NH); 3350 (NH); 3000 (=CH, CH); 1640 (C=N); 1620-1450 (aromatic); 1265 (C=S); cm^{-1}. (figure 15)

^{1}H-NMR ($CDCl_3$, d_5-pyrimidine): (figure 34)

Analysis for $C_{12}H_{11}N_5S_2$:

Calcd. C = 49.81 H = 3.83 N = 24.20

Found C = 49.98 H = 3.79 N = 23.76

Attempt to synthesize 5-Phenyl-3-phenylaminoisothiazolo-[3,4-d]pyrimidine-4-imine-6(7H)-thione

To a solution of 0.4325 gm (0.002 mole) 3-amino-4-cyanao-5-phenylaminoisothiazole in 10 ml pyridine, 0.2268 gm (0.002 mole) of phenyl isothiocyanate was added. The

solution was refluxed for 3 hours. After pyridine was distilled, the separated solid was collected and washed with acetone. The collected product was dried and finally, recrystallised with pyridine. However the product obtained was suspected to be 5,7-diphenyl-3-aminoisothiazolo[5,4-d]-pyrimidine-4-imine-6-thione. The total yield was 0.5694 gm (81.0%) , mp over 260 ^{o}C.

IR (KBr): 3450, 3400 (NH_2); 3200 (NH); 1750 (C=N); 1650-1450 (aromatic); 1250 (C=S); 775, 740 (monosubstituted aromatic) cm^{-1}. (figure 16)

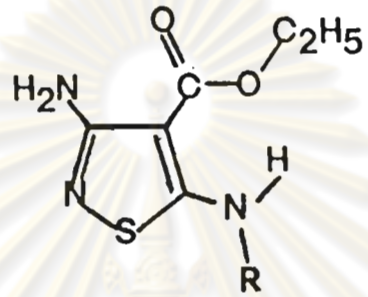
^{1}H-NMR ($CDCl_3$, d_5-pyridine) : (figure 35)

Analysis for $C_{17}H_{13}N_5S_2$:

Calcd. C = 58.09 H = 3.73 N = 19.93

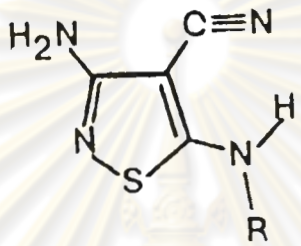
Found C = 58.09 H = 3.69 N = 19.56

TABLE 1: PHYSICAL DATA OF 3-AMINO-4-CARBETHOXY-5-SUBSTITUTEDAMINOISOTHIAZOLES



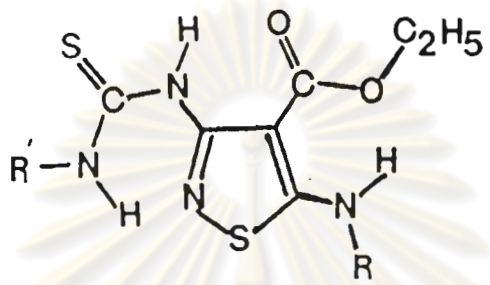
No.	R	Molecular Formular	Yield (%)	M.P. (°C)
1	CH_3	$C_7H_{11}N_3O_2S$	50.0	153-155
2	C_6H_5	$C_{12}H_{13}N_3O_2S$	89.5	159-160

TABLE 2: PHYSICAL DATA OF 3-AMINO-4-CYANO-5-SUBSTITUTEDAMINOISOTHIAZOLES



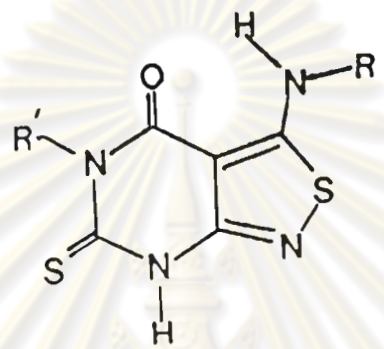
No.	R	Molecular formular	Yield (%)	M.P. (°C)	Elemental Analysis Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
3	CH_3	$C_5H_6N_4S$	60.0	255-256	38.95	38.70	3.92	3.85	36.33	36.29
4	C_6H_5	$C_{10}H_8N_4S$	40.0	231-232	55.54	55.42	3.72	3.69	25.91	25.90

TABLE 3: PHYSICAL DATA OF N-SUBSTITUTED-N'-(4-CARBETHOXY-5-SUBSTITUTEDAMINO-3-ISOTHIAZOLYL)-THIOUREAS



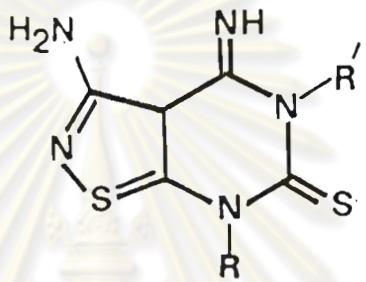
No.	R	R'	Molecular Formular	Yield (%)	M.P. (°C)	Elemental Analysis Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
5	CH_3	CH_3	$C_9H_{14}N_4O_2S_2$	18.0	133-135	39.40	39.36	5.14	5.20	20.42	20.26
6	CH_3	C_6H_5	$C_{14}H_{16}N_4O_2S_2$	55.0	169-170	49.98	49.91	4.79	4.79	16.65	16.51
7	C_6H_5	CH_3	$C_{14}H_{16}N_4O_2S_2$	32.0	148-150	49.98	50.32	4.79	4.78	16.65	16.44
8	C_6H_5	C_6H_5	$C_{19}H_{18}N_4O_2S_2$	72.0	131-132	57.26	57.19	4.55	4.55	14.06	14.00

TABLE 4: PHYSICAL DATA OF 5-SUBSTITUTED-3-SUBSTITUTEDAMINOISOTHIAZOLO[3,4-d]PYRIMIDINE-4-ONE-6(7H)-THIONE



No.	R	R′	Molecular Formular	Yield (%)	M.P. (°C)	Elemental Analysis Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
9	CH_3	CH_3	$C_7H_8N_4OS_2$	50.0	>260	36.82	36.89	3.51	3.56	24.55	24.09
10	CH_3	C_6H_5	$C_{12}H_{10}N_4OS_2$	32.0	>260	49.63	49.29	3.45	3.33	19.30	18.84
11	C_6H_5	CH_3	$C_{12}H_{10}N_4OS_2$	46.0	>260	49.63	49.32	3.45	3.38	19.30	18.84
12	C_6H_5	C_6H_5	$C_{17}H_{12}N_4OS_2$	30.0	>260	57.93	57.63	3.41	3.31	15.90	15.79

TABLE 5: PHYSICAL DATA OF 5,7-DISUBSTITUTED-3-AMINOISOTHIAZOLO[5,4-d]PYRIMIDINE-4-IMINE-6-THIONE



No.	R	R'	Molecular Formular	Yield (%)	M.P. (°C)	Elemental Analysis Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
13	CH_3	CH_3	$C_7H_9N_5S_2$	50.0	>260	36.99	36.97	3.99	3.94	30.81	30.56
14	CH_3	C_6H_5	$C_{12}H_{11}N_5S_2$	72.0	>260	49.81	49.84	3.83	3.79	24.20	23.80
15	C_6H_5	CH_3	$C_{12}H_{11}N_5S_2$	65.0	>260	49.81	49.98	3.83	3.79	24.20	23.76
16	C_6H_5	C_6H_5	$C_{17}H_{13}N_5S_2$	81.0	>260	58.09	58.09	3.73	3.69	19.93	19.56

CHAPTER IV

DISCUSSION

In this research, the isothiazolopyrimidine derivatives were synthesized in 3 steps:

1. Synthesis of 3,5-Diamino isothiazoles
2. Synthesis of N-Isothiazolyl thioureas
3. Cyclization of N-Isothiazolyl thioureas

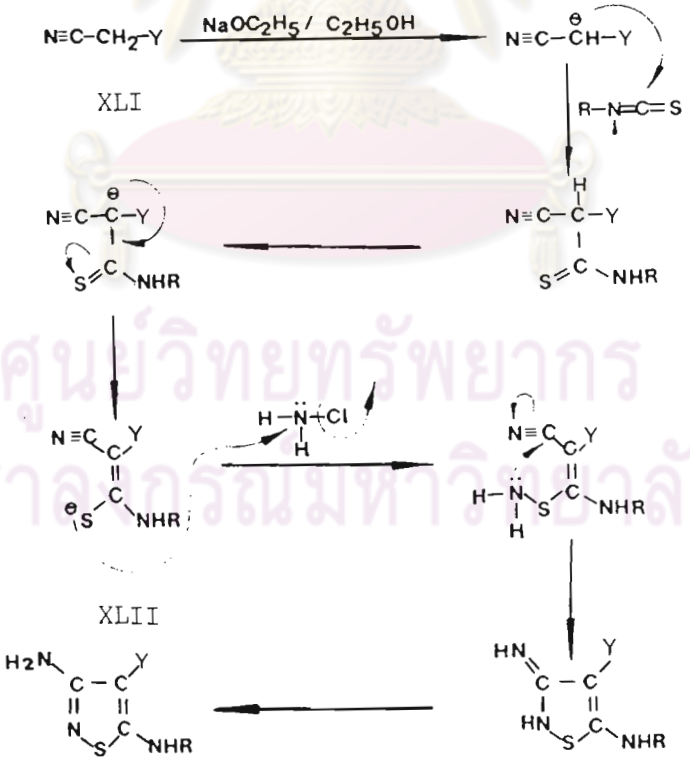
SYNTHESIS OF 3,5-DIAMINO ISOTHIAZOLES

The synthesis of 3,5-diaminoisothiazoles by the aminative cyclization of 3-amino-3-mercaptoacrylonitriles appeared to be the method of choice. The approach is particularly attractive because of the easy formation of 3-amino-3-mercaptoacrylonitriles from active methylene nitriles when reacting with isothiocyanates.

The reaction of the active methylene nitriles such as alkylcyanoacetate or malononitrile with isothiocyanate has usually been performed in the presence of an alkali metal alkoxide as the base.(49, 50, 51) The base promoted condensation of active methylene nitriles with isothiocyanates lead to the corresponding salts of 3-amino-3-mercaptoacrylonitrile intermediates which were

subsequently employed without isolation, to react with chloramine. Thus, the generation of 3-amino-3-mercapto-acrylonitrile salts <u>in situ</u> and cyclization with aqueous chloramine constitutes the synthesis of 3,5-diaminoisothiazole derivatives.

In the present work, two 3-amino-4-carbethoxy-5-substituted isothiazoles (XLIII, , Y = CO_2Et) were prepared by using ethylcyanoacetate (XLI, Y = CO_2Et) in sodium ethoxide and the corresponding isothiocyanate. The resulting intermediate 3-amino-3-mercaptoacrylonitrile(XLII, Y = CO_2Et) was then cyclized with chloramine. Scheme 2 describes the synthesis of this type of compound.



Scheme 2: Mechanism of Reaction of 3,5-Diaminoisothiazole Synthesis
Y = CO_2Et, CN

The methylene hydrogens adjacent to the carbonyl or the cyano functional groups have considerably greater acidity than the hydrocarbon analogs. That is attributed to the ability of the carbonyl and cyano group to delocalize the negative charge of the conjugate base. The conjugate base of carbonyl compound is the important "enolate anion", which is also involved in the base catalyzed enolization process. The formation of enolate anion of ethylcyano-acetate is illustrated by using sodium ethoxide in absolute ethanol. The functional group, carbonyl and cyano groups, may stabilize carbanion by delocalization of the negative charge.

Anion formed can be added to the electrophilic carbon center of the isothiocyanate leading to an intermediate, 3-amino-3-thioacrylonitrile, (Y = CO_2Et) which also possess active methylene group. Electron-delocalization in the intermediate finally give 3-amino-3-mercaptoacrylonitrile (XLII, Y = CO_2Et).

Chloramine as source of an amino group, was generated by the reaction of ammonium hydroxide and sodium hypochlorite in low temperature. The nucleophilic substitution at electrophilic nitrogen atom of chloramine by nucleophilic sulfur atom of 3-amino-3-mercaptoacrylonitrile produces the amine. Following by nucleophilic reaction of amino group at carbon atom of cyano functional group to give 3,5-diaminoisothiazoles

(XLIII, Y = CO_2Et).

The ^{1}H-NMR spectrum in figure 19 and IR Spectrum in figure 1 indicated that the obtained product was 3-amino-4-carbethoxy-5-methylaminoisothiazole. The ^{1}H-NMR spectrum indicated ethoxy group at 1.37 ppm (t, J = 7, 3H, $-CH_2-CH_3$) and 4.35 ppm (q, J = 7, 2H, $-O-CH_2-CH_3$); methyl group at 2.93 ppm (d, J = 5.4, 3H, $-NH-CH_3$); secondary amine group at 7.79 ppm (br, 1H, $-NH-CH_3$) and primary amine group at 5.48 ppm (s, 2H, isothiazole-NH_2). The IR spectrum indicated primary amine group at 3500, 3380 cm^{-1}; secondary amine at 3330 cm^{-1}; ester gruop at 1680 (C=O stretching) and 1240 (C-O stretching) cm.-1

The ^{1}H-NMR spectrum in figure 20 and IR spectrum in figure 2 indicated that the obtained product was 3-amino-4-carbethoxy-5-phenylaminoisothiazole. The ^{1}H-NMR spectrum indicated ethoxy group at 1.43 ppm (t, J = 7, 3H, $-CH_2-CH_3$) and 4.37 ppm (q, J = 7, 2H, $-O-CH_2-CH_3$); phenyl group at 7.18 ppm (m, 5H, $-NH-C_6H_5$); secondary amine group at 9.97 ppm (br, 1H, $-NH-C_6H_5$) and primary amine group at 5.48 ppm (s, 2H, isothiazole-NH_2). The IR spectrum indicated primary amine group at 3500, 3300 cm^{-1}; secondary amine group at 3180 cm^{-1}; ester group at 1660 cm^{-1} (C=O stretching) and at 1220 (C-O stretching) cm.

Two 3-amino-4-cyano-5-substituted aminoisothiazoles (XLIII, Y = CN) were prepared by using malononitrile (XLI, Y = CN) in sodium ethoxide and the corresponding

nitrile (XLII, Y = CN), was then cyclized with chloramine. The mechanism of reaction was similar to that of 3-amino-4-carbethoxy-5-substitited aminoisothiazole synthesis.

The ^{1}H-NMR spectrum in figure 22 and IR spectrum in figure 4 showed that 3-amino-4-cyano-5-methylaminoisothiazole was the obtained product. The ^{1}H-NMR spectrum indicated methyl group at 3.80 ppm (d,J = 5.4, 3H,-NH-CH_3); secondary amine group at 8.09 ppm (br, 1H, -NH-CH_3) and primary amine group at 5.96 ppm (s, 2H, isothiazole-NH_2). The IR spectrum indicated primary amine group at 3450, 3360 cm^{-1}; secondary group at 3200 cm^{-1} and cyano group at 2200 cm^{-1}.

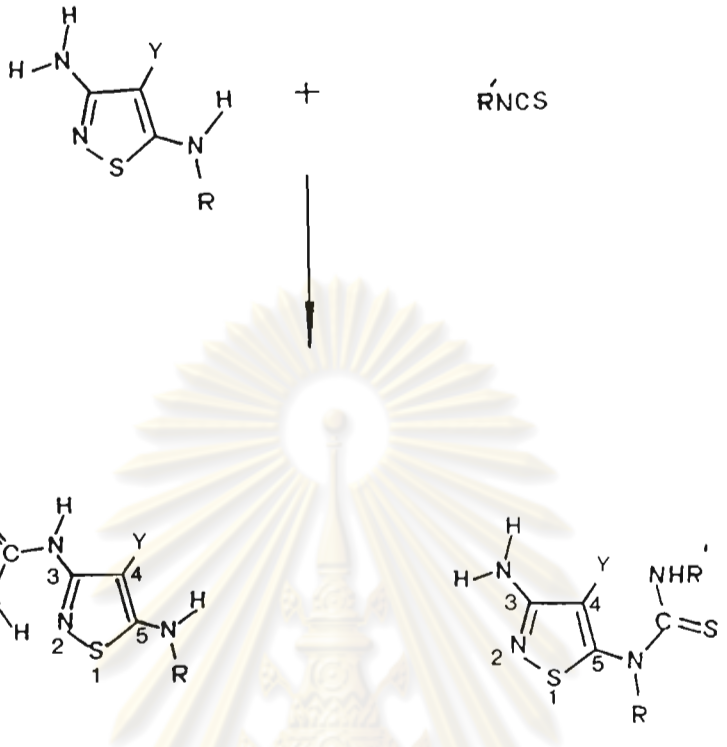
The ^{1}H-NMR spectrum in figure 21 and IR spectrum in figure 4 showed that 3-amino-4-cyano-5-phenylaminoisothiazole was the obtained product. The ^{1}H-NMR spectrum indicated phenyl group at 7.29 ppm (m, 5H, -NH-C_6H_5); secondary amine group at 10.12 ppm (br, 1H, -NH-C_6H_5) and primary amine group at 5.81 ppm (s,2H,isothiazole-NH_2). The IR spectrum indicated primary amine group at 3450, 3340 cm^{-1}; secondary amine group at 3200 cm^{-1} and cyano group at 2200 cm^{-1}.

SYNTHESIS OF N-ISOTHIAZOLYL THIOUREAS

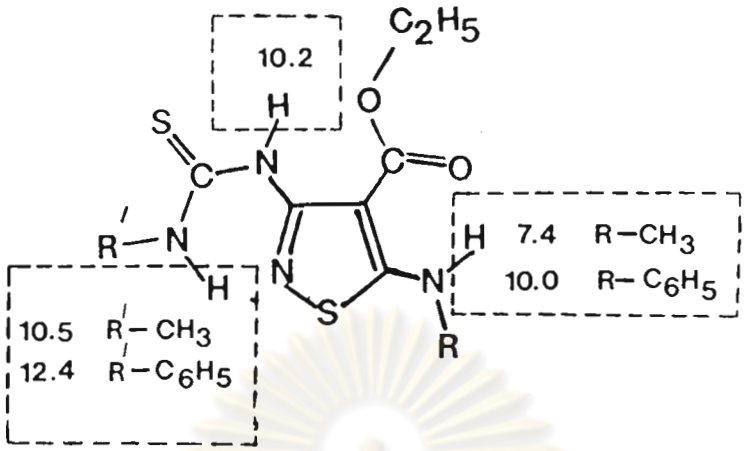
Here, the 3-aminoisothiazole derivatives were used to form the thioureas with methyl isothiocyanate or phenyl isothiocyanate in various conditions. The reaction was carried out in pyridine or toluene, when ethanol was used as the solvent the thiocarbamate was obtained due to the reaction of ethanol and isothiocyanate. Formation of the thiocarbamate was observed when carrying the reaction of phenyl isothiocyanate and aminoisothiazole in ethanol, the product phenylthiocarbamate was isolated.(NMR spectrum was shown in figure 35.)

3,5-Diaminoisothiazole derivatives (XLIII) were transformed to the N-isothiazolylthioureas (XLIV , XLV) by the reaction of the free amine bases, 3-amino-4-carbethoxy-5-methylaminoisothiazole, 3-amino-4-carbethoxy-5-phenylaminoisothiazole, 3-amino-4-cyano-5-methylaminoisothiazole or 3-amino-4-cyano-5-phenylaminoisothiazole with methyl or phenyl isothiocyanate in pyridine under refluxing condition. There are two possible sites of reaction on the aminoisothiazoles which were represented as follows.

1. The substitution reaction occured at 3 position
2. The substitution reaction occured at 5 position

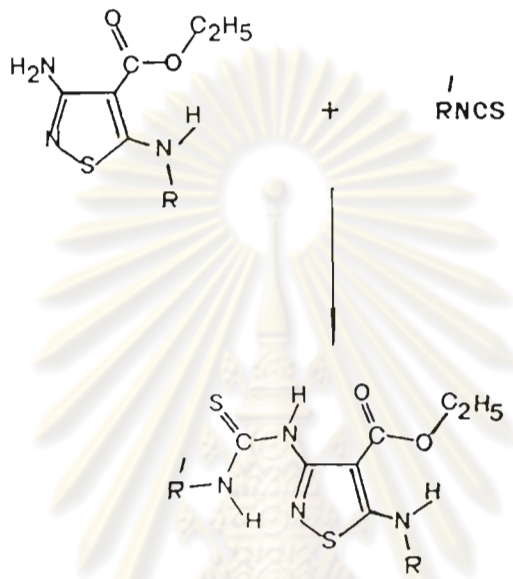


The ^{1}H-NMR spectra of the product obtained ; N-methyl-N$'$-(4-carbethoxy-5-methylamino-3-isothiazolyl)-thiourea (figure 23), N-phenyl-N$'$-(4-carbethoxy-5-methylamino-3-isothiazolyl)-thiourea (figure 24), N-methyl-N$'$-(4-carbethoxy-5-phenylamino-3-isothiazolyl)-thiourea(figure 25), and N-phenyl-N$'$-(4-carbethoxy-5-phenylamino-3-isothiazolyl)-thiourea indicated that the addition reaction occured at 3 position, not at 5 position, of isothiazole rings. Three N-H protons were assigned as follows.

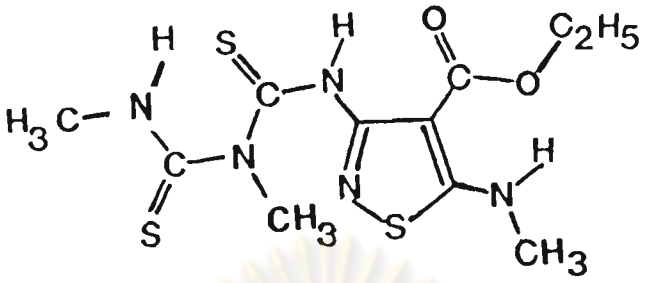


In the series of reaction of 4-carbethoxy isothiazoles with the isothiocyanates, the reaction occured mainly at 3 position. This can be explained that because of the bulk of carbethoxy group, the intramolecular hydrogen bonding between the hydrogen of amine group at 5 position and the carbonyl oxigen of the carbethoxy group at 4 positon form stainless six-membered ring conformation, which also cause rigid steric hindrance and electron delocalization, gives the nucleophilic group at 3 position more readily reactive than that at 5 positon.

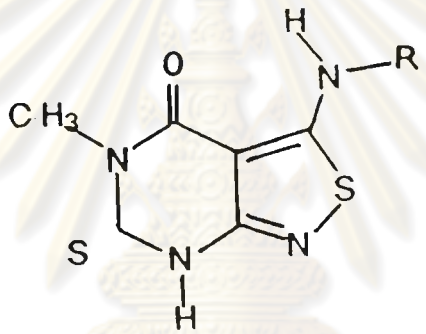
The mechanism of substitution reaction occured at 3 position can be represented as follow.



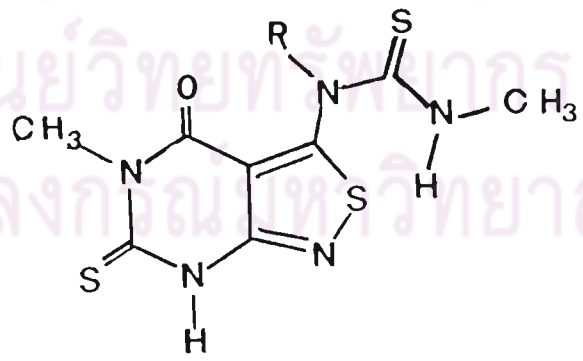
In the case of the reaction of 3,5-diaminoisothiazole derivatives with methyl isothiocyanate , Which R group is less bulky, three by-products, the thiobiuret (XLV), the cyclization products (XLVI) and the cyclizationproducts having thiocarbamoyl group at 5 position (XLVII) were isolated when excess of methyl isothiocyanate was used.



XLV



XLVI



XLVII

where R = CH_3, C_6H_4

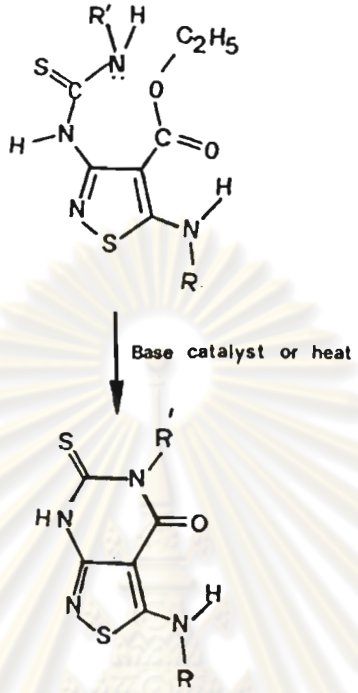
CYCLIZATION OF N-ISOTHIAZOLYL THIOUREAS

The N-isothiazolyl thioureas can be cyclized in present of catalyst, such as acid catalyst, base catalyst, chemical catalyst or heat.

However Isothiazolopyrimidines cannot be obtained by refluxing N-isothiazolyl thioureas in toluene, toluene with zinc chloride, xylene, xylene with zinc chloride or dilute sodium hydroxide solution.

Isothiazolopyrimidines were obtained via the base and heat catalytic cyclization of N-isothiazolyl thioureas. Thus, 5-methyl-3-methylamino isothiazolo[3,4-d]pyrimidine-4-one-6(7H)-thione , 5-phenyl-3-methylamino isothiazolo-[3,4-d]pyrimidine-4-one-6(7H)-thione, 5-methyl-3-phenyl-amino isothiazolo[3,4-d]pyrimidine-4-one-6(7H)-thione and 5-phenyl-3-phenylamino isothiazolo[3,4-d]pyrimidine-4-one-6(7H)-thione were readily obtained respectively form N-methyl-N′- (4-carbethoxy-5-methylamino-3-isothiazolyl)-thiourea, N-phenyl-N′-(4-carbethoxy-5-methylamino-3-iso-thiazolyl)-thiourea, N-methyl-N′-(4-carbethoxy-5-phenyl amino-3-isothiazolyl)-thiourea and N-phenyl-N′-(4-carbethoxy-5-phenylamino-3-isothiazolyl)-thiourea after a short period of refluxing with methyl or phenyl isothiocyanate in pyridine medium. N-Isothiazolyl thioureas were smoothly cyclized to yield the target compounds which were in general, difficult to purify by crystallization.

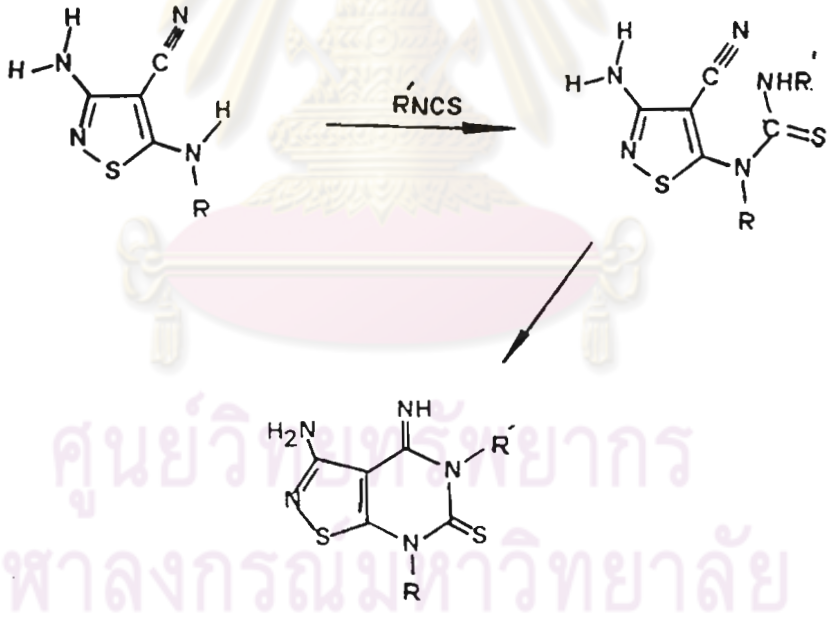
The following reaction describes the synthesis of this type of compounds.



Because of the poor solubility of the obtained product, the poor ^{1}H-NMR spectra were obtained. However all IR spectra and elemental analysis confirmed the structures of required products. The obtained products, 5-methyl-3-methylamino isothiazolo[3, 4 - d]pyrimidine -4-one-6(7H)-thione showed peaks in ^{1}H-NMR spectrum (figure 27) for methyl group at 3-amino position at 2.9 ppm (doublet), the peak for methyl group at 5 position at 3.81 ppm (singlet), the peak of amine group at 3 and 7 position were not observed (this structure should show the peak of amine groups at 3 and 7 position at about 7.2-7.8 and 10.0 ppm respectively) and the IR spectrum (figure 9) showed two secondary amine groups at 3280, 3200 cm^{-1}, carbonyl group

at 1660 cm^{-1} two methyl groups at 1398, 1360 cm^{-1} and thione group at 1250 cm^{-1} ; 5-phenyl-3-methylamino isothiazolo[3,4-d]pyrimidine-4-one-6(7H)-thione showed peaks in ^{1}H-NMR spectrum (figure 28) for methyl group at 3-amino position at 2.89 ppm, the peak for phenyl group at 5 position at 7.3 ppm (multiplet), the peak for amine group at 3 position at 7.79 ppm (this structure should show the peak of amine group at 7 position at about 10 ppm) and the IR spectrum (figure 10) showed two secondary amine groups at 3400, 3330 cm^{-1} , carbonyl group at 1660 cm^{-1} , methyl group at 1360 cm^{-1} , thione group at 1200 cm^{-1} and monosubstituted aromatic peak at 770, 740 cm^{-1} ; 5-methyl-3-phenylaminoisothiazolo[3, 4-d]pyrimidine-4-one-6(7H)-thione showed peaks in ^{1}H-NMR spectrum (figure 29) for methyl group at 5 position at 3.65 ppm (singlet), the peak for phenyl group at 3 position at 7.4 ppm (multiplet), the peak of amino groups at 3 and 7 position at 10.12 ppm and the IR spectrum (figure 11) showed two secondary amines at 3400, 3200 cm^{-1} , carbonyl group at 1640 and thione group at 1240 cm^{-1} ; and 5-phenyl-3-phenylaminoisothiazolo-[3,4-d]-pyrimidine-4-one-6(7H)-thione showed peaks in ^{1}H-NMR spectrum (figure 30) for two phenyl groups at at 7.3 ppm, amine groups at 10.28 ppm and the IR spectrum (figure 12) showed two secondary amines at 3400, 3300 cm^{-1} , carbonyl group at 1668 cm^{-1} , the thione group at 1200 cm^{-1} and monosubstituted aromatic peak at 775, 740 cm.

With the reaction of 3-amino-4-cyano-5-phenyl or 3-amino-4-cyano-5-methyl aminoisothiazole and methyl or phenyl isothiocyanate in pyridine, the corresponding thioureas were not observed, however the obtained products were concluded as 5,7-dimethyl-3-aminoisothiazolo[5, 4-d]-pyrimidine-4-imine-6-thione, 5-phenyl-7-methyl-3-aminoisothiazolo[5,4-d]pyrimidine-4-imine-6-thione, 5-methyl-7-phenyl-3-aminoisothiazolo[5 , 4-d]pyrimidine-4-imine-6-thione and 5,7-diphenyl-3-aminoisothiazolo[5,4-d]pyrimidine-4-imine-6-thione. Respectively the following reaction present the synthesis of this type of compounds.



The obtained products, 5,7-dimethyl-3-aminoisothiazolo[5,4-d]pyrimidine-4-imine-6-thione showed peaks in ^{1}H-NMR spectrum (figure 31) for methyl group at 5 and 7 position at 3.79 (singlet) and 2.84 (singlet) respectively,

the peak for primary amine and imine showed at 5.01 ppm because of the chemical exchange between NH group of this product with OH group of water and the IR spectrum (figure 13) showed primary amine at 3450, 3350 cm^{-1}, secondary amine at 3250 cm^{-1} and thione group at 1250 cm^{-1}; 5-phenyl-7-methyl-3-aminoisothiazolo[5 , 4-d]pyrimidine-4-imine-6(7H)-thione showed peaks in ^{1}H-NMR spectrum (figure 32) for methyl group at 7 position at 2.95 ppm (singlet), phenyl group at 5 position 7.2 ppm (multiplet), the peak for primary amine and imine showed at 6.9 ppm because of the chemical exchange between NH group of this product and OH group of water and the IR spectrum (figure 14) showed thione group at 1250 cm^{-1} and monosubstituted peak at 775, 740 cm^{-1}; 5-methyl-7-phenyl-3-aminoisothiazolo-[5,4-d]pyrimidine-4-imine-6-thione showed peaks in ^{1}H-NMR spectrum (figure 33) for methyl group at 5 position at 3.86 ppm (singlet), phenyl group at 7 position at 7.3 ppm (multiplet), the peak for primary amine and imine showed at 4.58 ppm because of the chemical exchange between NH group of this product and OH group of water and the IR spectrum (figure 15) showed primary amine at 3450, 3350 cm^{-1} and thione group at 1650 cm^{-1}; and 5-phenyl-7-phenylaminoiso-thiazolo[5,4-d]pyrimidine-4-imine-6-thione showed peaks in ^{1}H-NMR spectrum (figure 34) for two phenyl group at 5 and 7 position at 7.3 ppm (multiplet), the primary amine and imine group showed at 6.9 ppm because of the chemical exchange between NH group of this product and OH group of

water and the IR spectrum (figure 16) showed primary amine at 3450, 3400 cm^{-1} and thione group at 1250 cm^{-1} and monosubstituted aromatic at 775, 740 cm^{-1}. All elemental analyses of the products confirm the represented structures.

In case of reaction of 4-cyano isothiazole derivatives with the isothiocyanate, the adduct thiuorea trend to occur intermediate with the amino group at 5 position due to both steric and electronic effect. Sterically, there is no intramolecular hydrogen bonding between the NH at 5 position and the nitrile group at 4 position, this leaves the substituted amino group at 5 position freely rotate and attacking of the isothiocyanate group is possible, in addition, the nitrile group is less bulky than carbethoxy group. Electronically the amino group at 5 position is relatively more nucleophilic than that at 3 position due to the electron donating effect of the substituted alkyl or phenyl group. Once the adduct thiourea is formed, the cyclyization trend to proceed readily to a stainless six-membered ring products. Therefore, the thiourea intermediate was not observed in the reaction mixture. Eventhough the products obtained were logically concluded to be the isothiazolo[5,4-d]pyrimidine derivatives, more information on the identification and structural elucidation need to be performed for a complete conclusion.

CHAPTER V

CONCLUSION

Two series of isothiazolopyrimidine derivatives, 5-substituted-3-substituted aminoisothiazolo[3,4-d]pyrimidine-4-one-6(7H)-thiones and 5,7-disubstituted-3-aminoisothiazolo[5,4-d]pyrimidine-4-imine-6-thiones, were synthesized, starting from the reaction of 3,5-diaminoisothiazole derivatives and isothiocyanates to yield the N-isothiazolyl thioureas which were then cyclized by heating to yield the products, 5-methyl-3-methyl aminoisothiazolo[3,4-d]pyrimidine-4-one-6(7H)-thione, 5-phenyl-3-methyl aminoisothiazolo [3,4-d]pyrimidine-4-one-6(7H)-thione, 5-methyl-3-phenyl aminoisothiazolo[3,4-d]pyrimidine -4 - one -6(7H)-thione, 5-phenyl-3-phenyl aminoisothiazolo[3,4-d]pyrimidine-4-one-6(7H)-thione, 5,7-dimethyl-3-aminoisothiazolo[5,4-d]pyrimidine-4-imine-6-thione,5-phenyl-7-methyl-3-aminoisothiazolo-[5,4-d]pyrimidine-4-imine-6-thione, 5-methyl-7-phenyl-3-aminoisothiazolo[5,4-d]pyrimidine - 4 -imine - 6 -thione, and 5,7-diphenyl-3-aminoisothiazolo[5,4-d]pyrimidine-4-imine-6-thione. During the synthesis, 8 intermetiates, 3-amino-4-carbethoxy-5-methylaminoisothiazole, 3-amino-4-carbethoxy-5-phenylaminoisothiazole, 3-amino-4-cyano-5-methylaminoisothiazole, 3-amino-4-cyano-5-phenylaminoisothiazole, N-methyl- N'-(4-carbethoxy-5-methylamino-3-isothiazolyl)-thiourea, N-phenyl-N'-(4-carbethoxy-5-methyl-

amino-3-isothiazolyl)-thiourea, N-methyl-N'-(4-carbethoxy-5-phenylamino-3-isothiazolyl)-thiourea and N-phenyl-N'-phenyl-(4-carbethoxy-5-phenylaminoisothiazolyl)-thiourea were also obtained.

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APPENDIX

FIGURE 1: THE IR SPECTRUM OF 3-AMINO-4-CARBETHCXY-5-METHYLAMINOISOTHIAZOLE

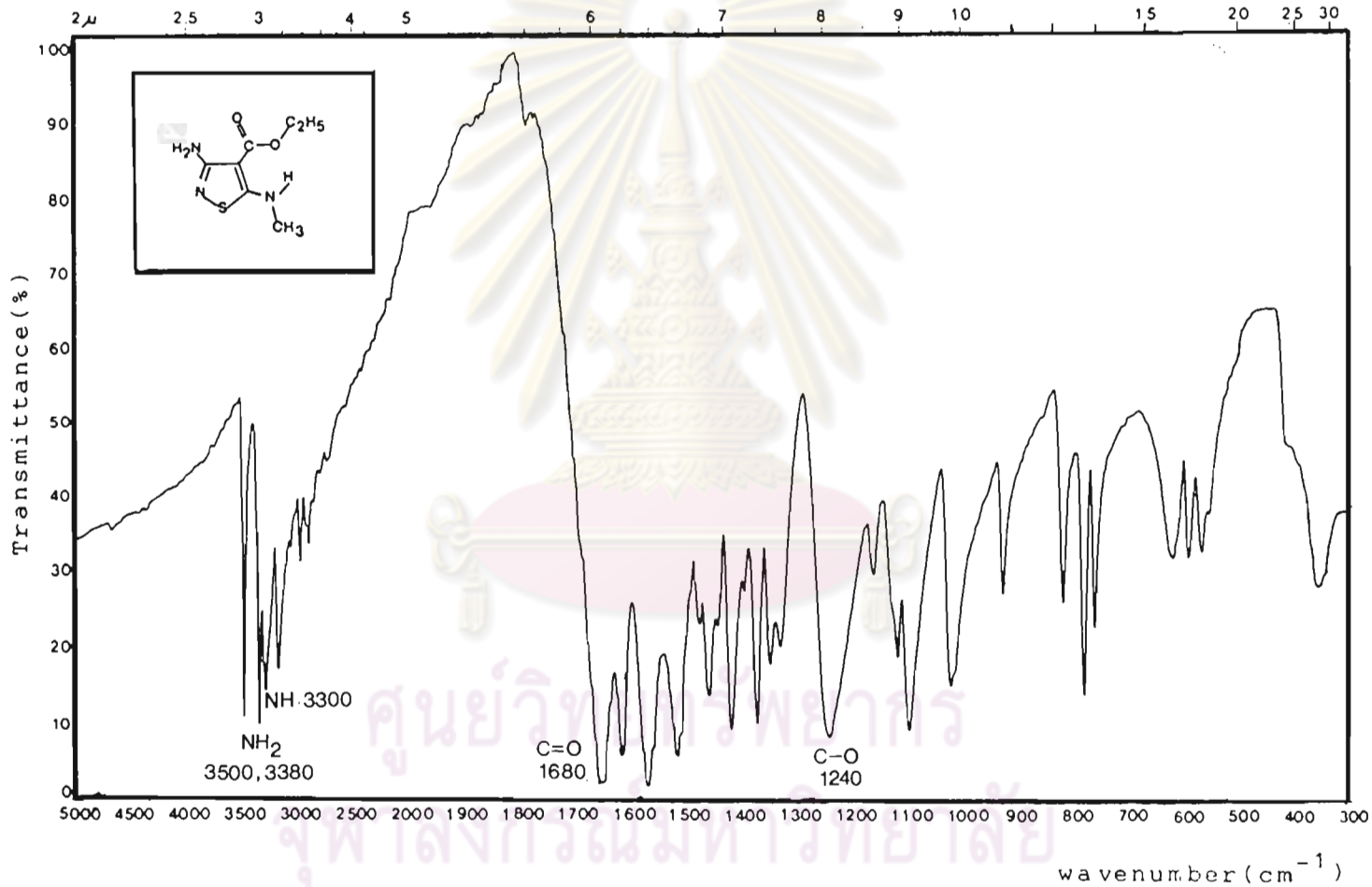


FIGURE 2: THE IR SPECTRUM OF 3-AMINO-4-CARBETHOXY-5-PHENYLAMINOISOTHIAZOLE

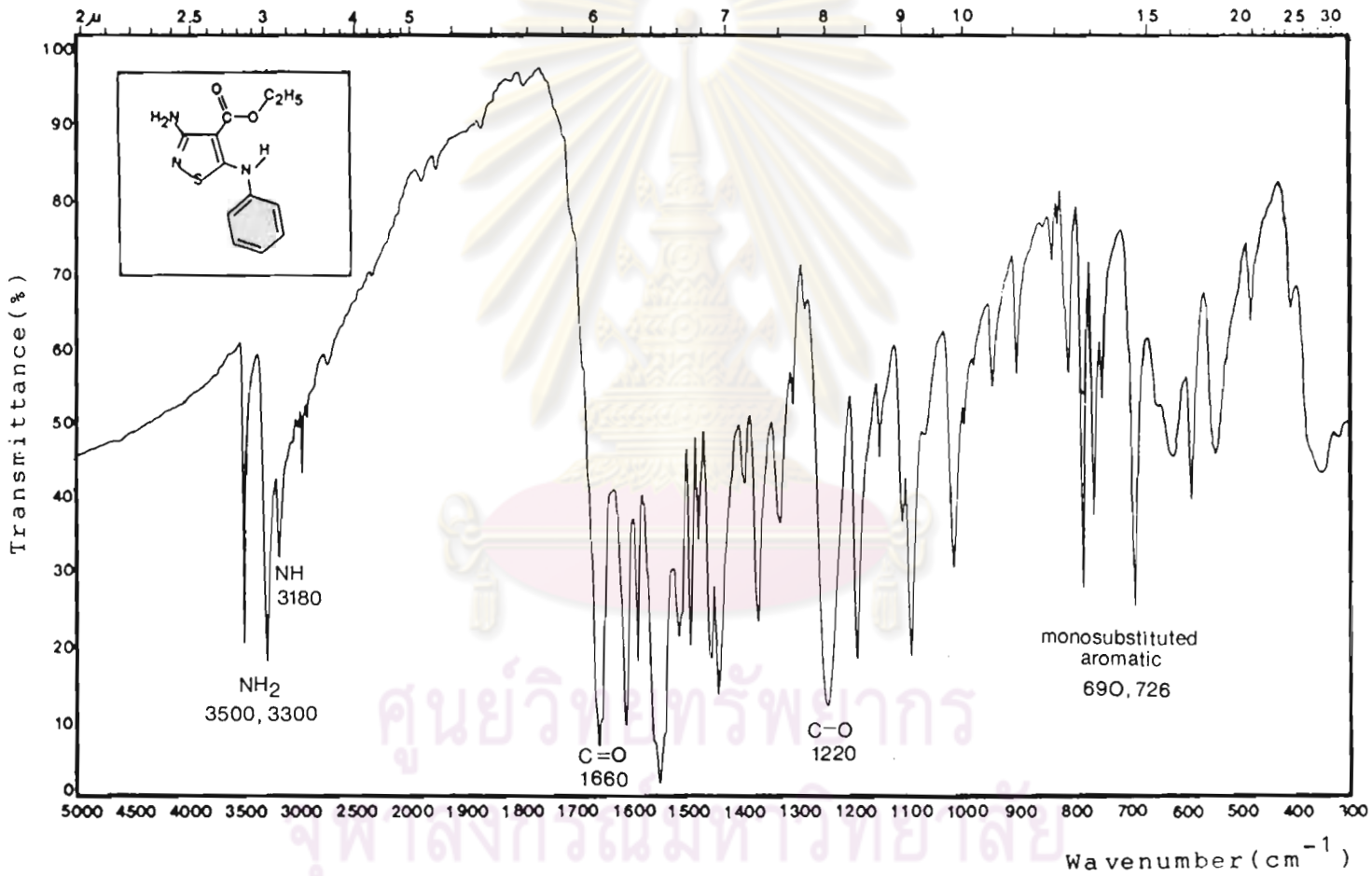


FIGURE 3: THE IR SPECTRUM OF 3-AMINO-4-CYANO-5-METHYLAMINOISOTHIAZOLE

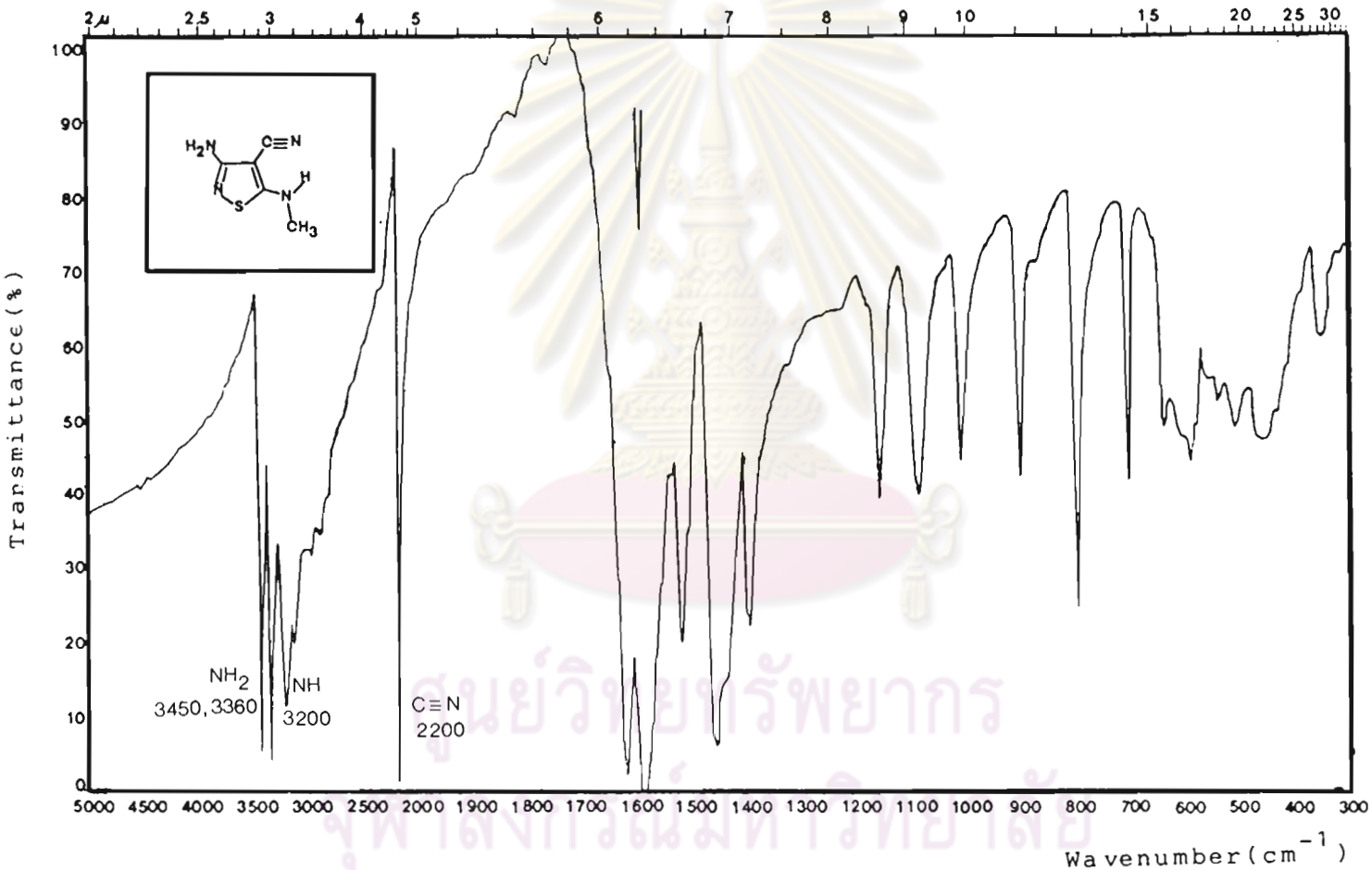


FIGURE 4: THE IR SPECTRUM OF 3-AMINO-4-CYANO-5-PHENYLAMINOISOTHIAZOLE

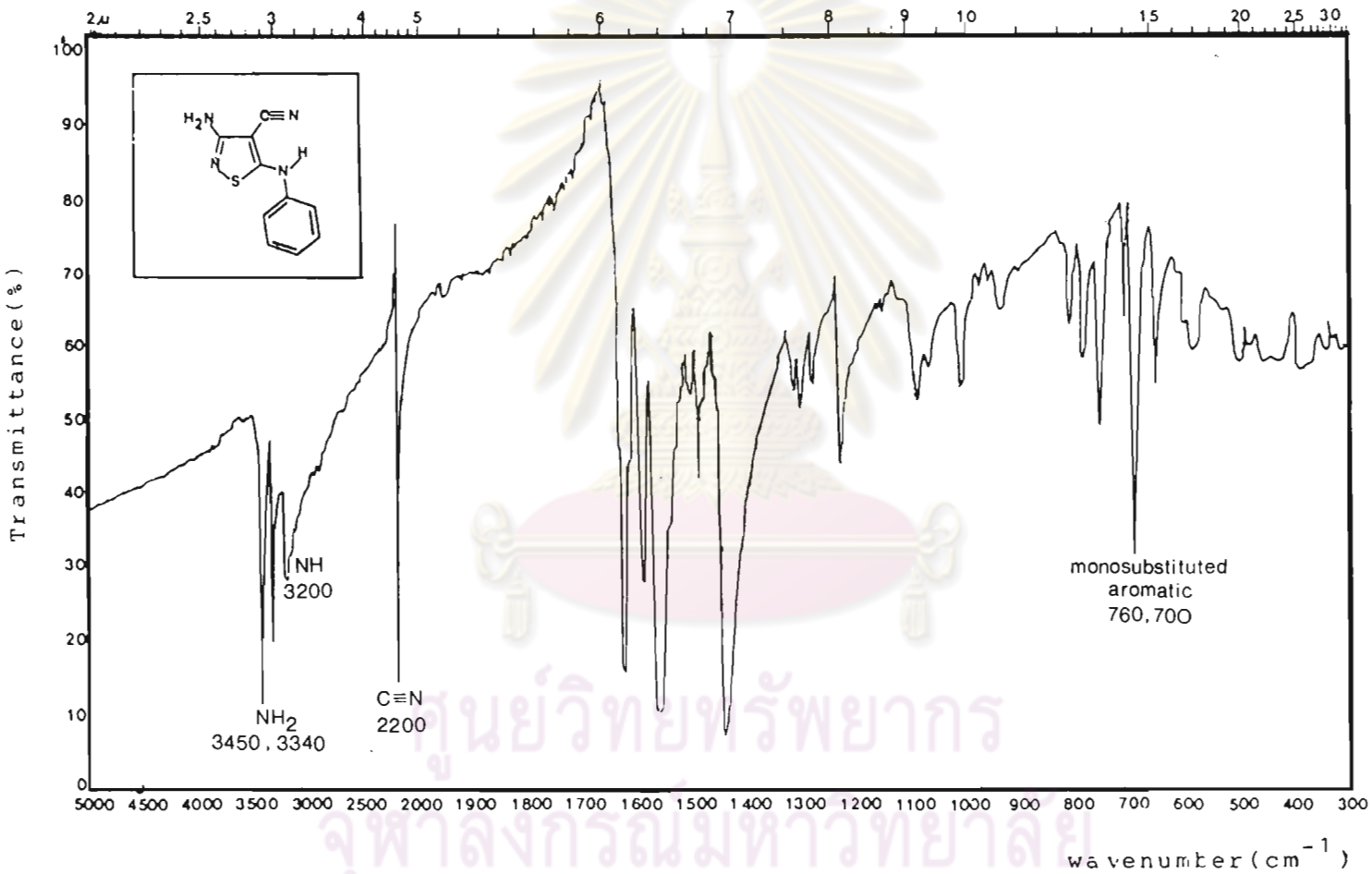


FIGURE 5: THE IR SPECTRUM OF N-METHYL-N'-(4-CARBETHOXY-5-METHYLAMINO-3-ISOTHIAZOLYL)-THIOUREA

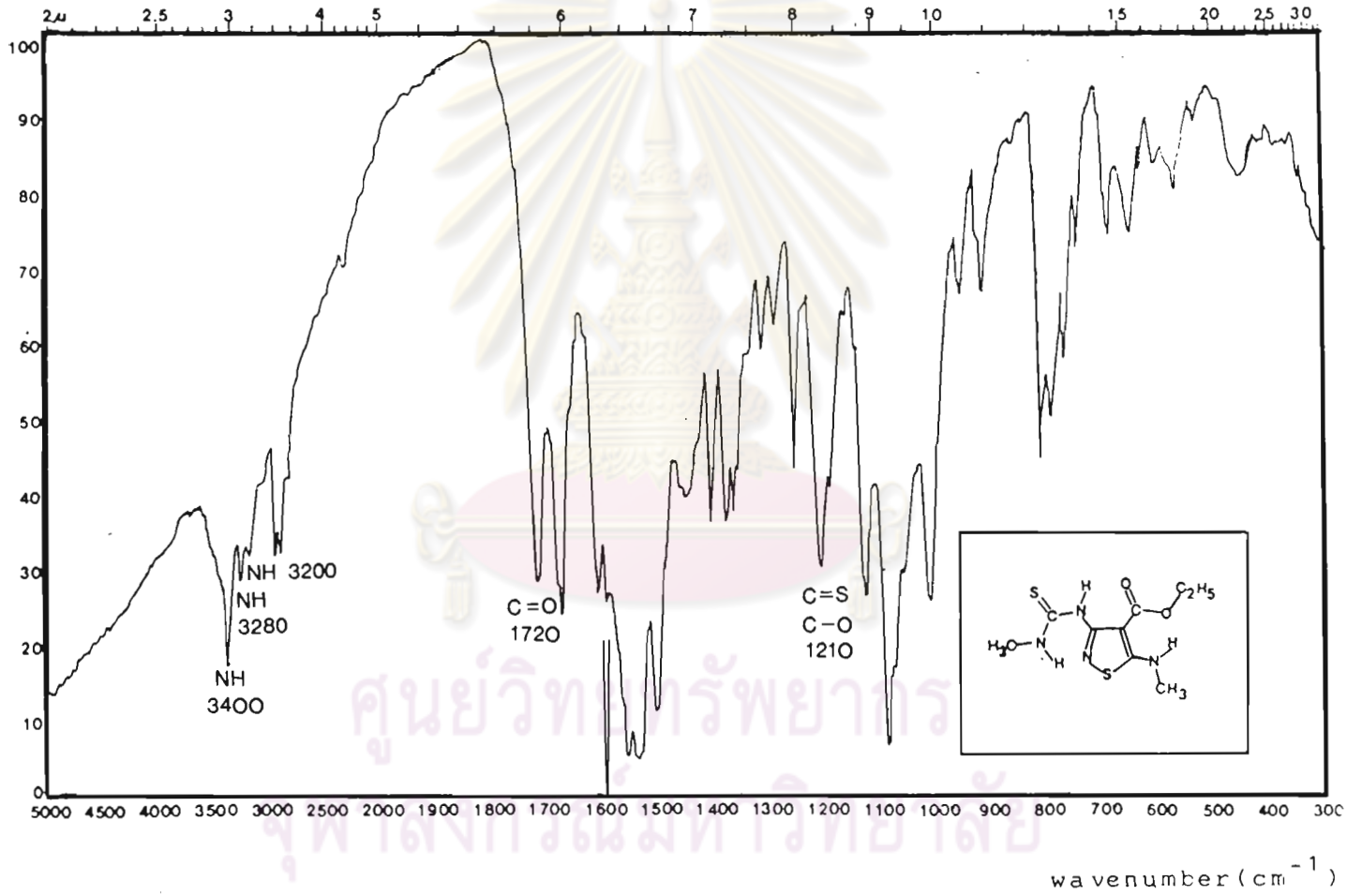


FIGURE 6: THE IR SPECTRUM OF N-PHENYL-N'-(4-CARBETHOXY-5-METHYLAMINO-3-ISOTHIAZOLYL)-THIOUREA

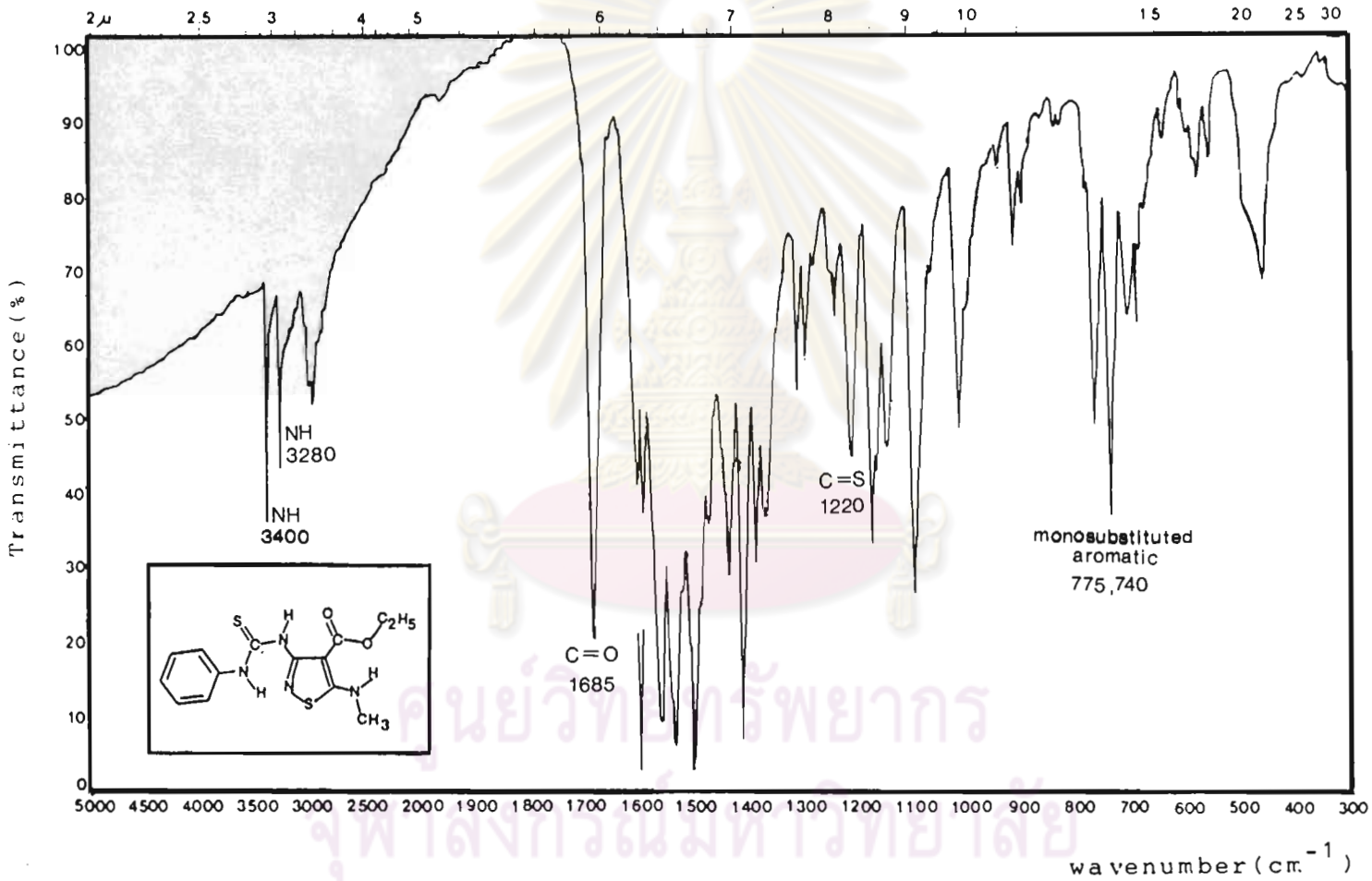


FIGURE 7: THE IR SPECTRUM OF N-METHYL-N'-(4-CARBETHOXY-5-PHENYLAMINO-3-AMINOISOTHIAZOLYL)-THIOUREA

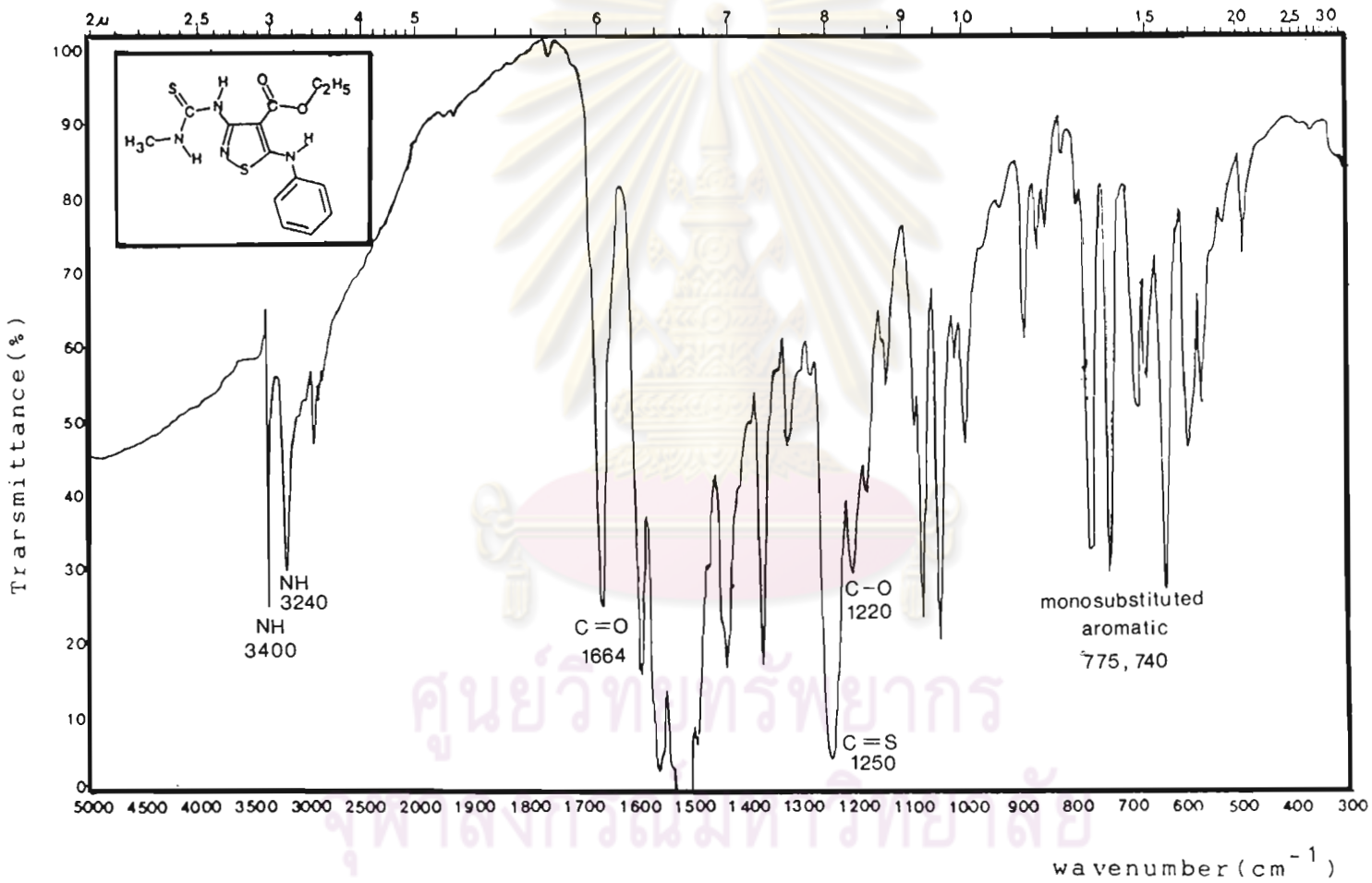


FIGURE 8: THE IR SPECTRUM OF N-PHENYL-N'-(4-CARBETHOXY-5-PHENYLAMINO-3-ISOTHIAZOLYL)-THIOUREA

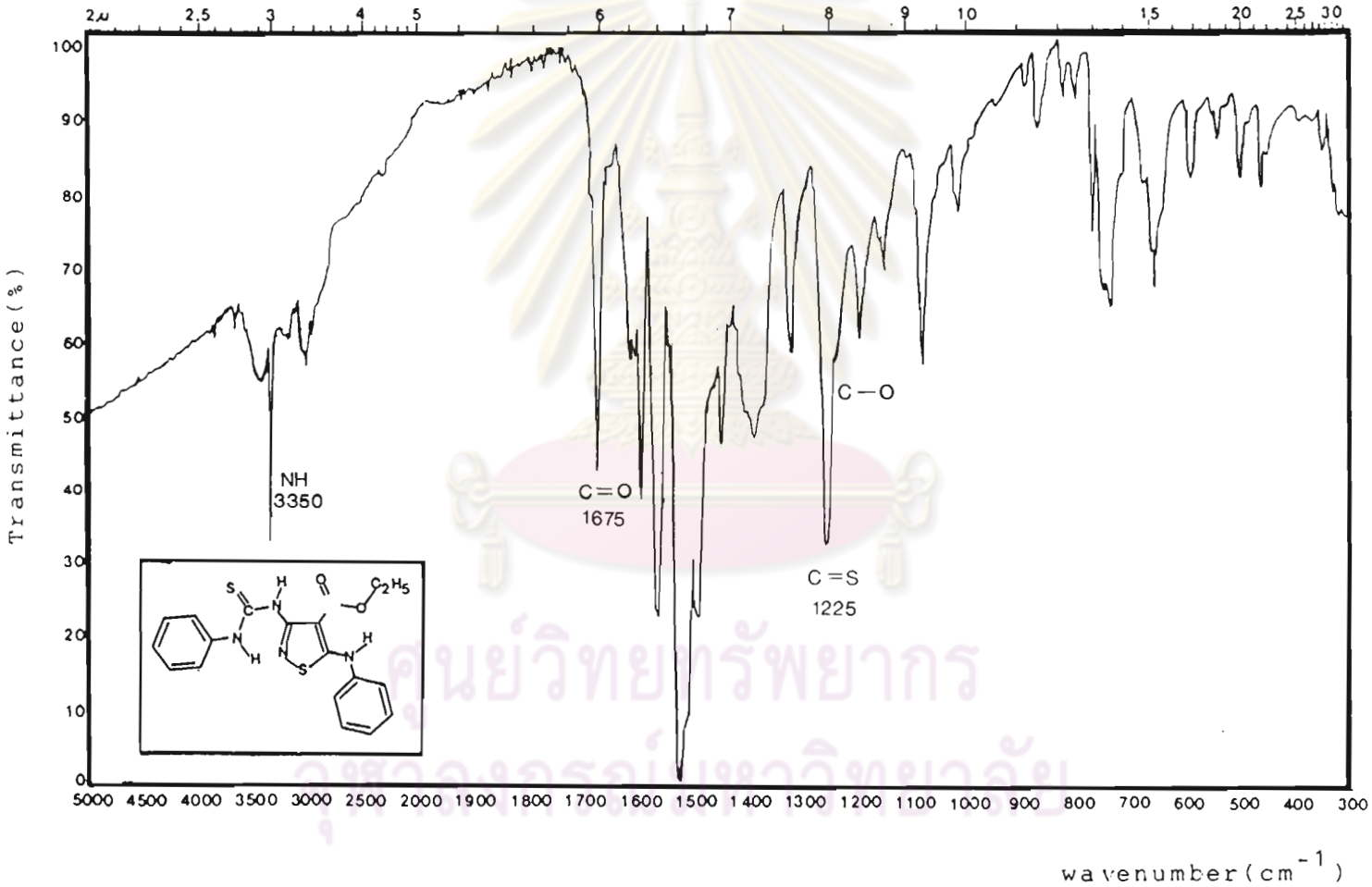


FIGURE 9: THE IR SPECTRUM OF 5-METHYL-3-METHYLAMINOISOTHIAZOLO[3,4-d] PYRIMIDINE-4-ONE-6(7H)-THIONE

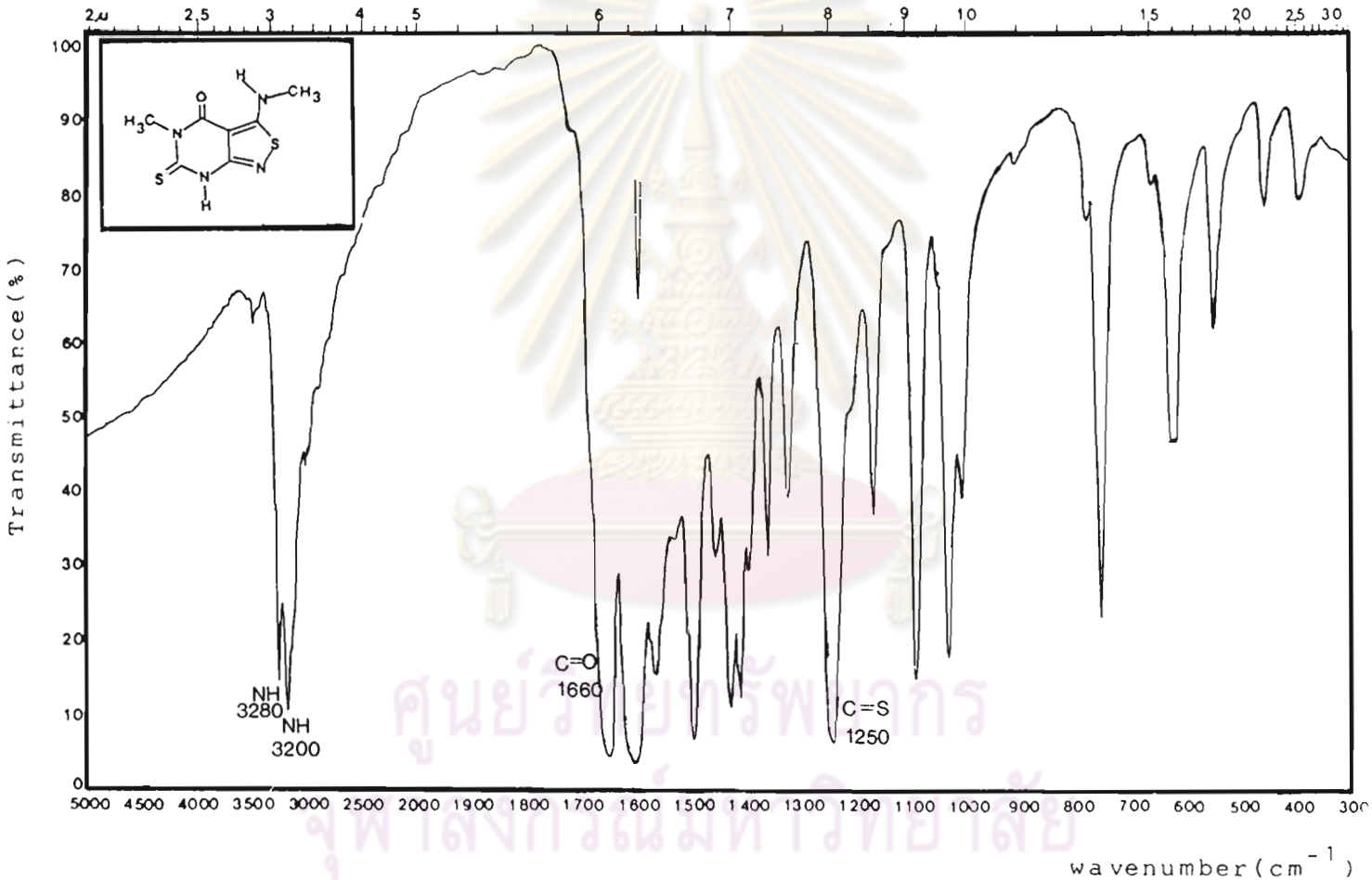


FIGURE 10: THE IR SPECTRUM OF 5-PHENYL-3-METHYLAMINOISOTHIAZOLO[3,4-d] PYRIMIDINE-6(7H)-THIONE

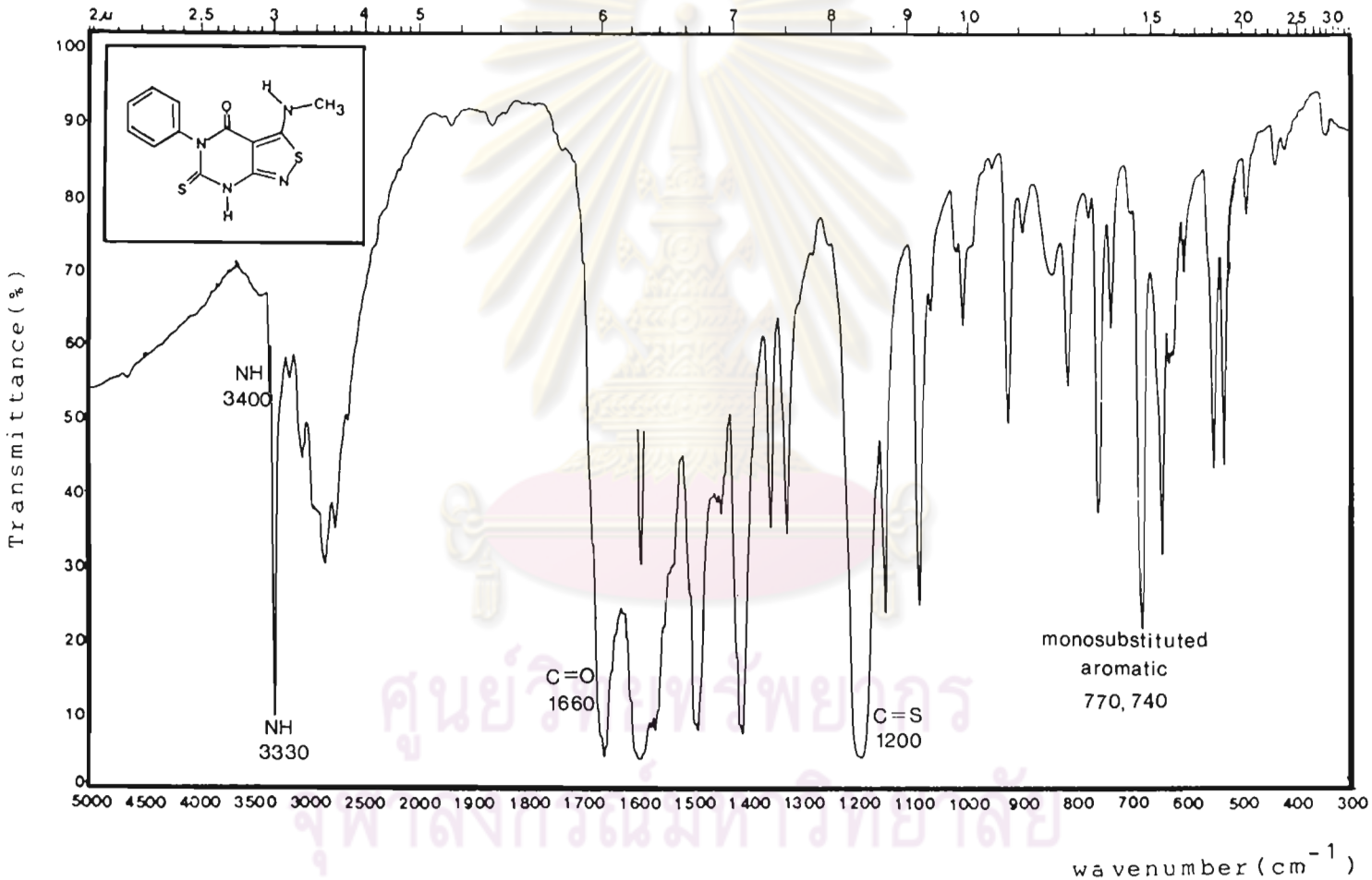


FIGURE 11: THE IR SPECTRUM OF 5-PHENYL-3-METHYLAMINOISOTHIAZOLO[3,4-d] PYRIMIDINE-6(7H)-THIONE

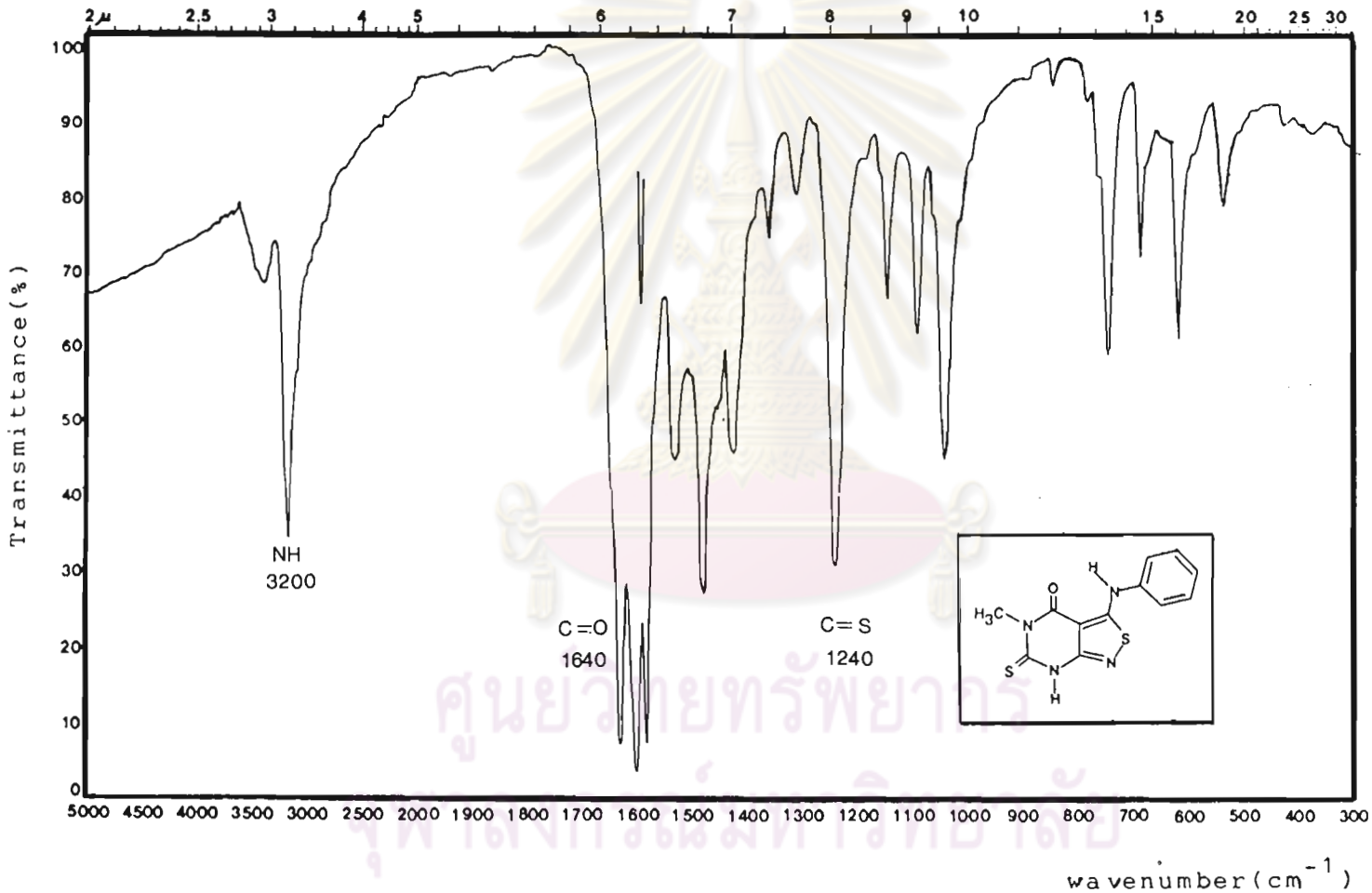


FIGURE 12: THE IR SPECTRUM OF 5-PHENYL-3-PHENYLAMINOISOTHIAZOLO[3,4-d] PYRIMIDINE-6(7H)-THIONE

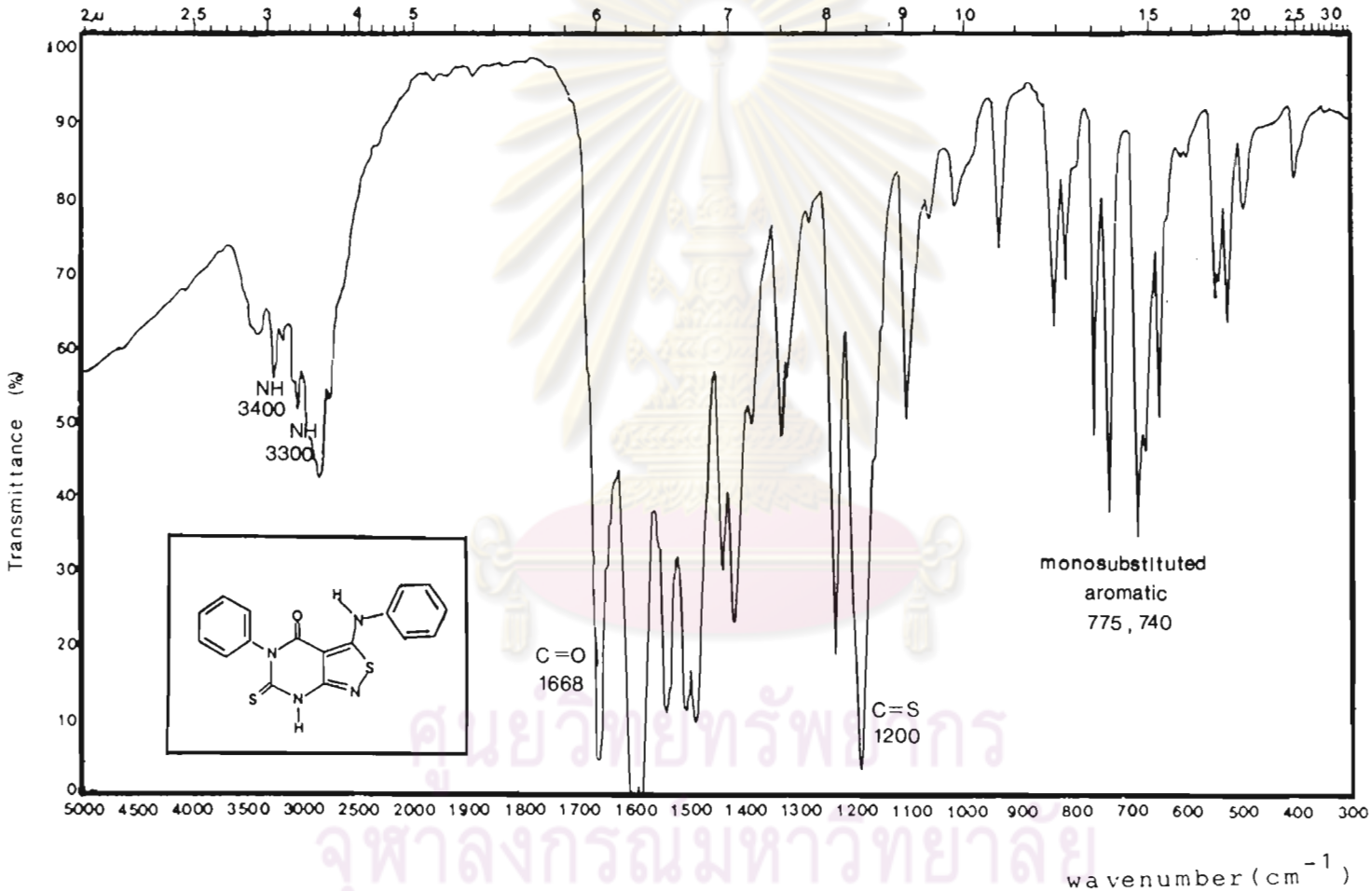


FIGURE 13: THE IR SPECTRUM OF 5,7-DIMETHYL-3-AMINOISOTHIAZOLO[5,4-d] PYRIMIDINE-4-IMINE-6-THIONE

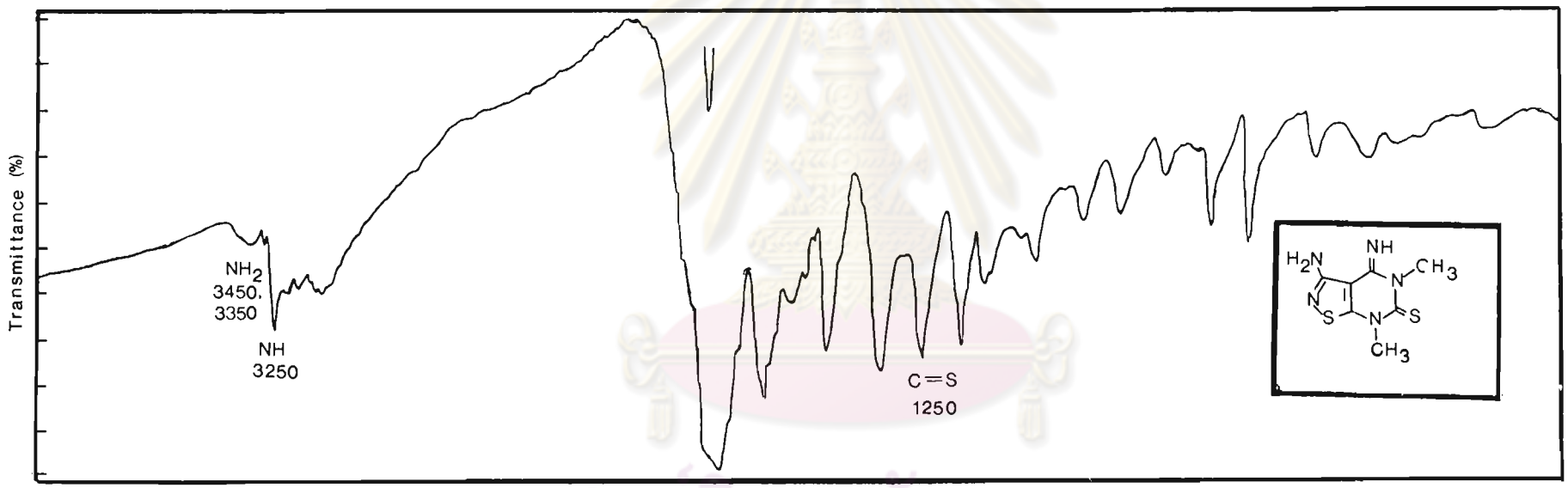


FIGURE 14: THE IR SPECTRUM OF 5-PHENYL-7-METHYL-3-AMINOISOTHIAZOLO[5,4-d] PYRIMIDINE-4-IMINE-6-THIONE

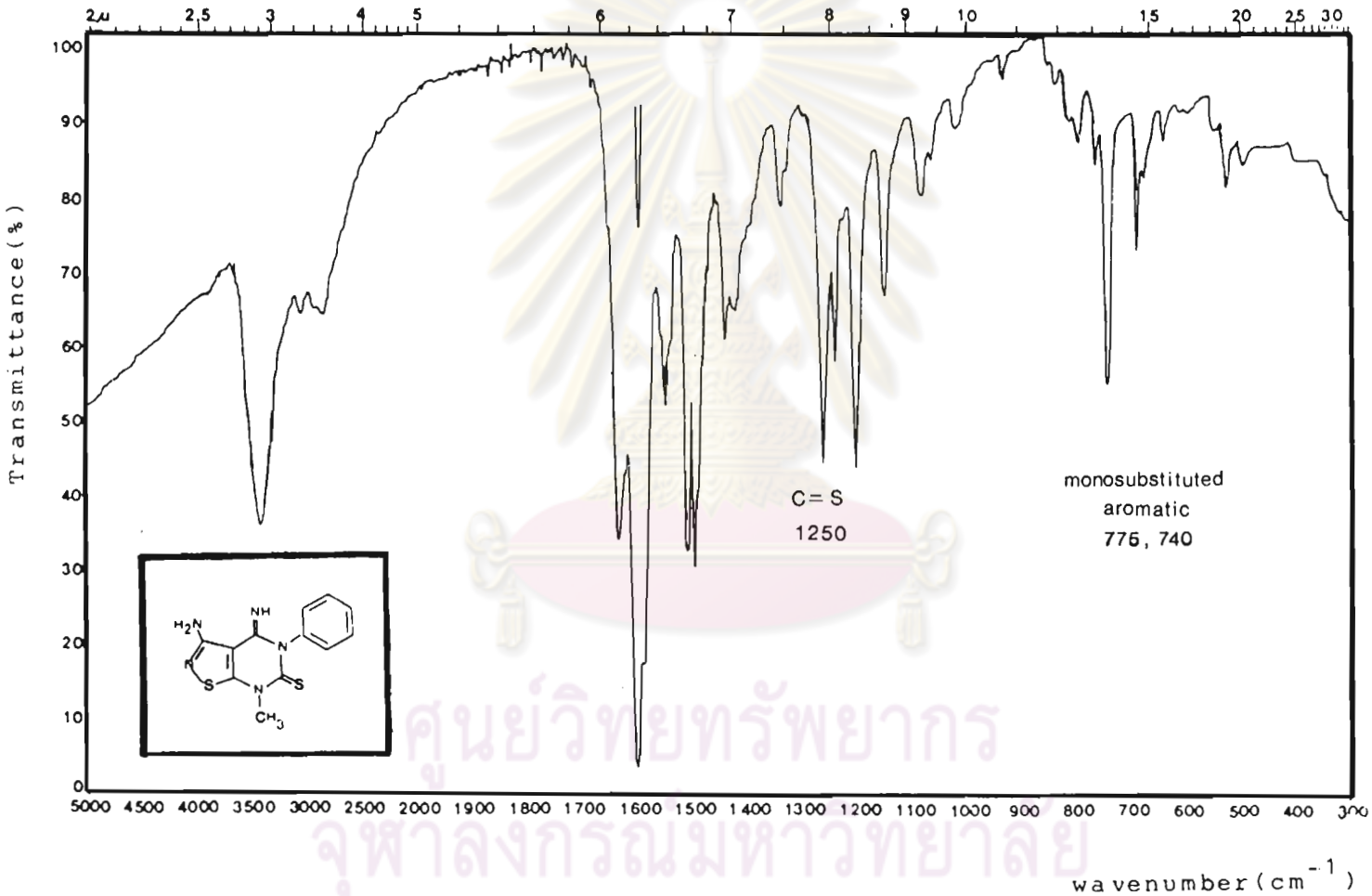


FIGURE 15: THE IR SPECTRUM OF 5-METHYL-7-PHENYL-3-AMINOISOTHIAZOLO[5,4-d] PYRIMIDINE-4-IMINE-6-THIONE

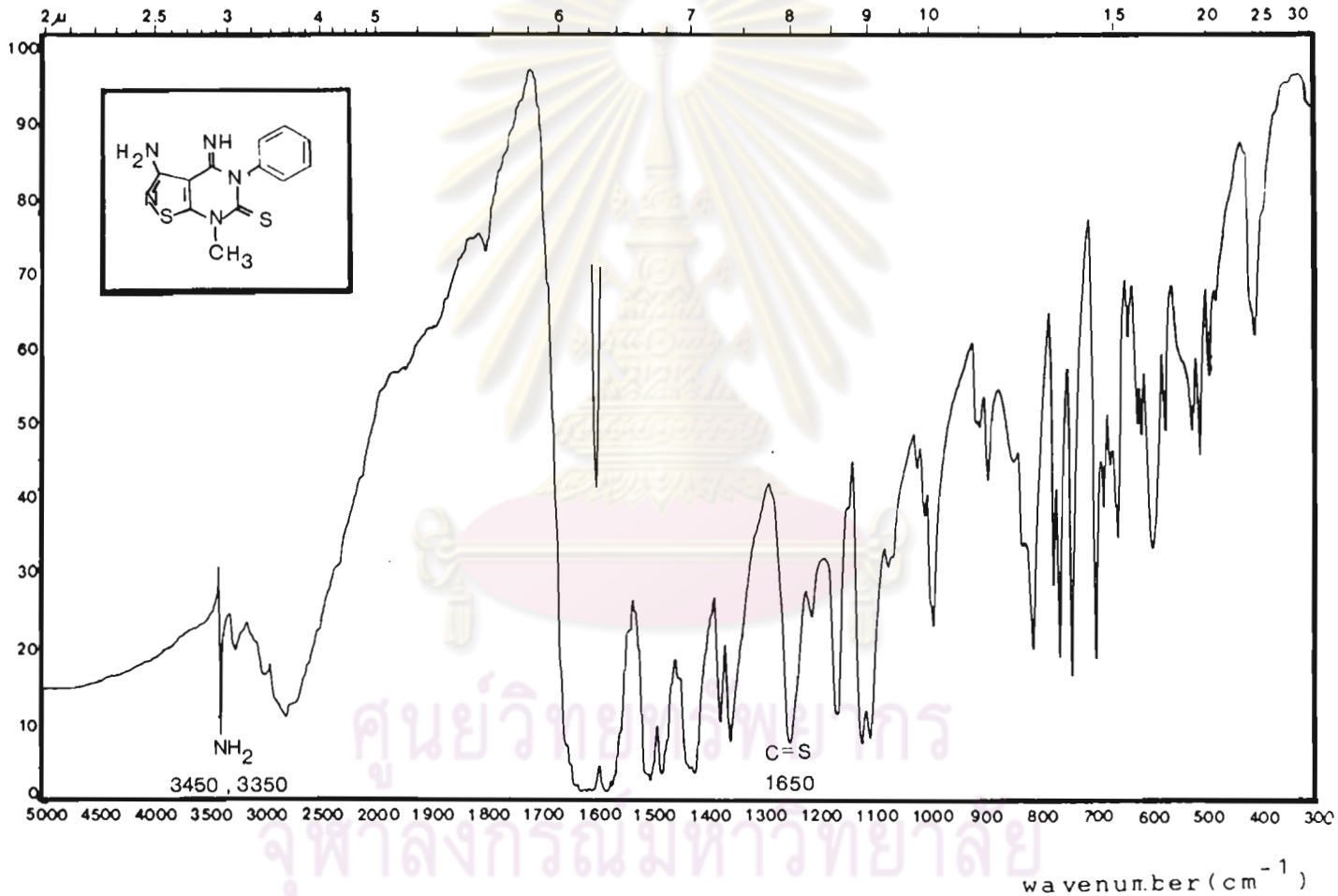


FIGURE 16: THE IR SPECTRUM OF 5,7-DIPHENYL-3-AMINOISOTHIAZOLO[5,4-d] PYRIMIDINE-4-IMINE-6-THIONE

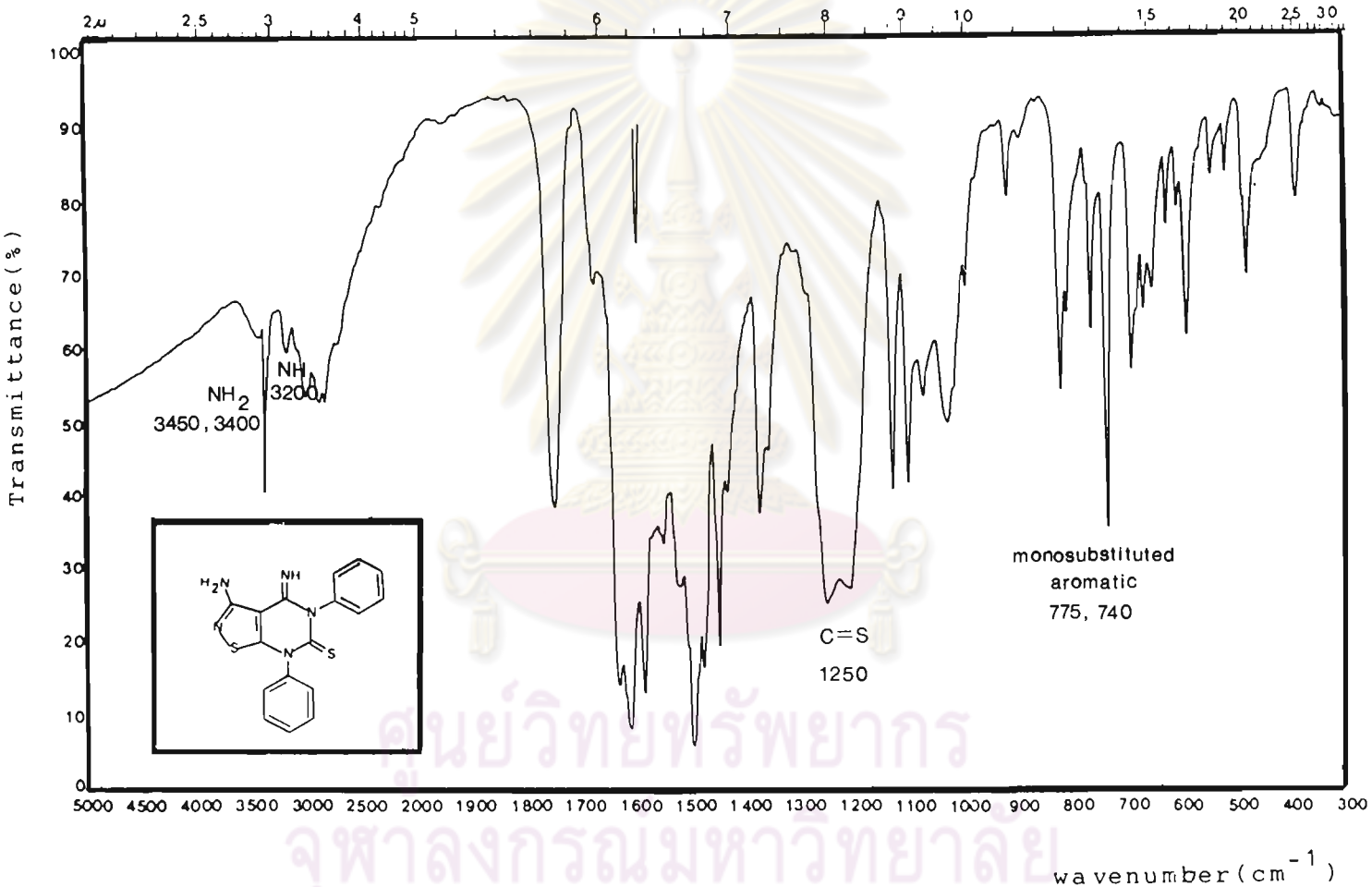


FIGURE 17: THE IR SPECTRUM OF N-METHYL-N'-(5-METHYL-3-METHYLAMINO ISOTHIAZOLO[3,4-d]PYRIMIDINE-4-ONE-6(7H)-THIONE)-THIOUREA

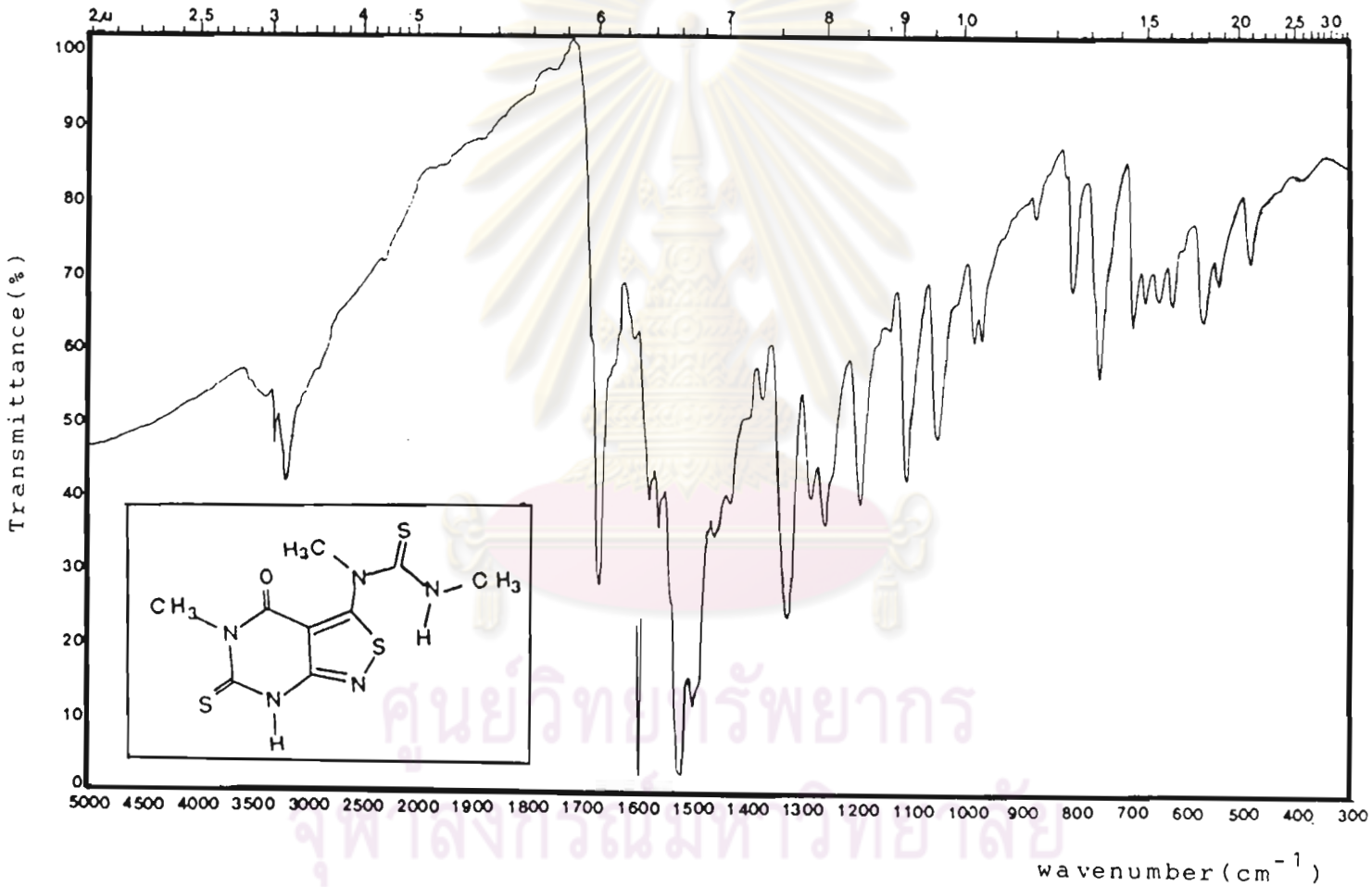
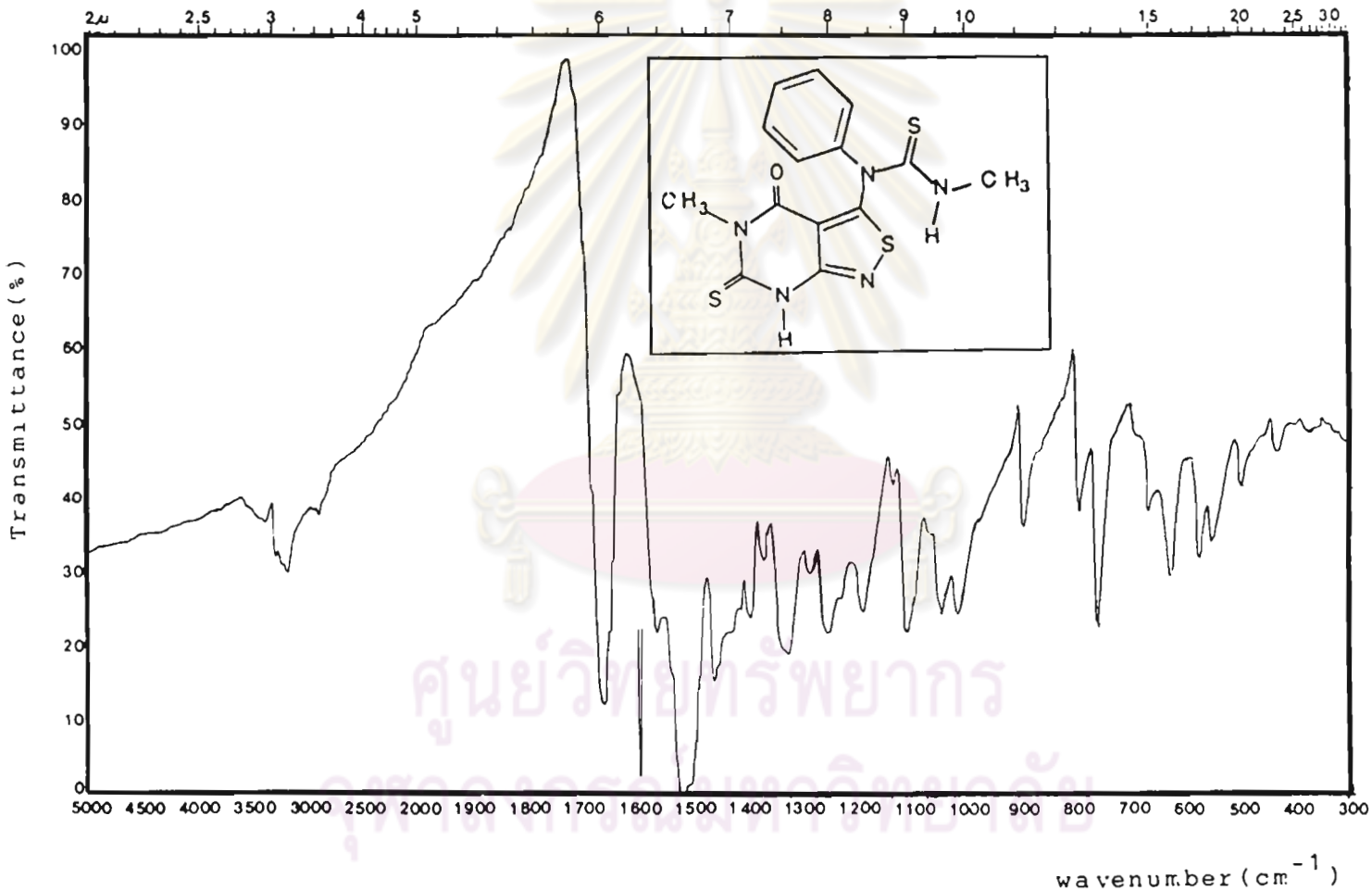


FIGURE 18: THE IR SPECTRUM OF N-METHYL-N'-(5-PHENYL-3-METHYLAMINO ISOTHIAZOLO[3,4-d]PYRIMIDINE-4-ONE-6(7H)-THIONE)-THIOUREA



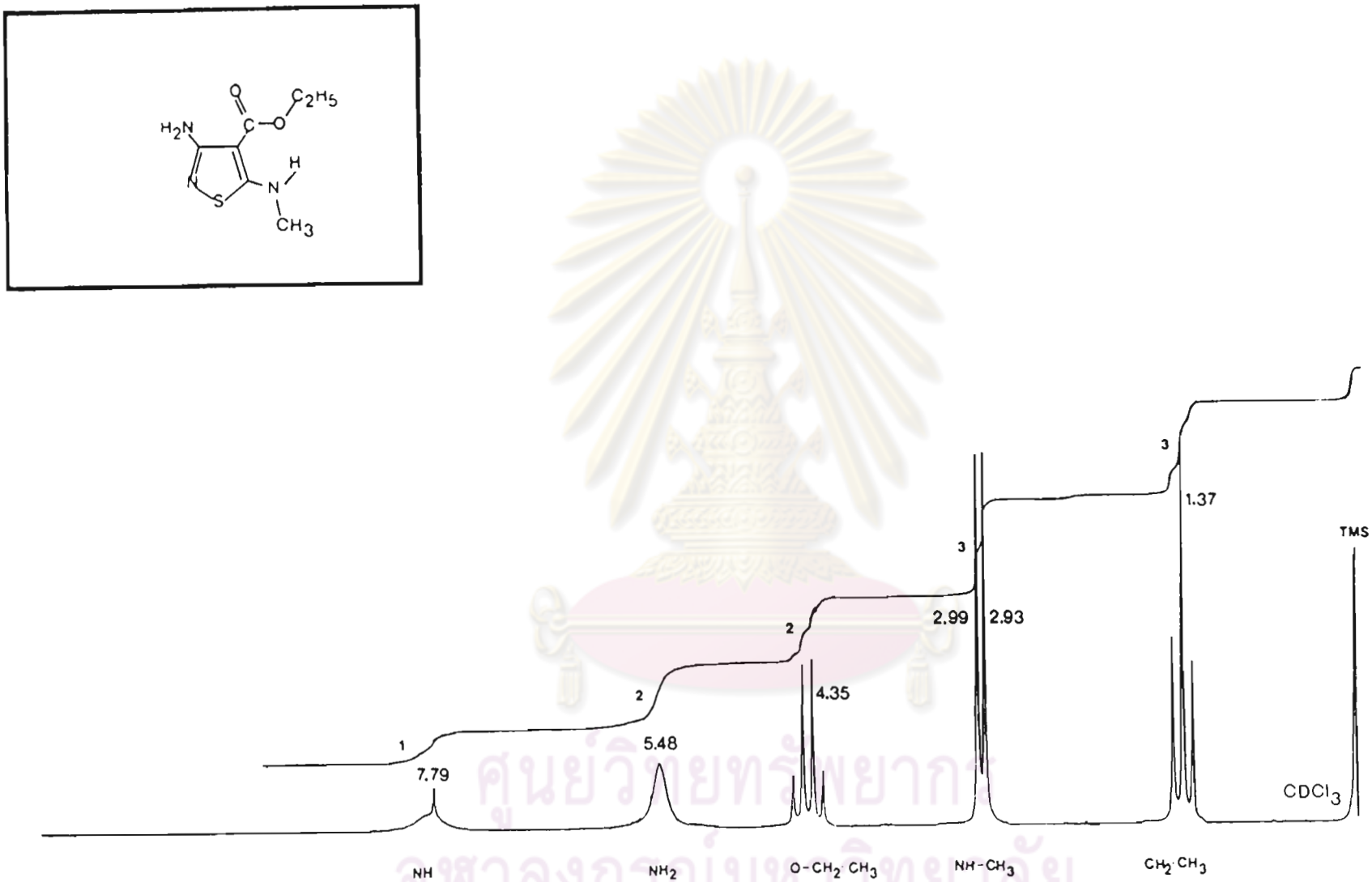


FIGURE 19: THE NMR SPECTRUM OF 3-AMINO-4-CARBETHOXY-5-METHYLAMINOISOTHIAZOLE

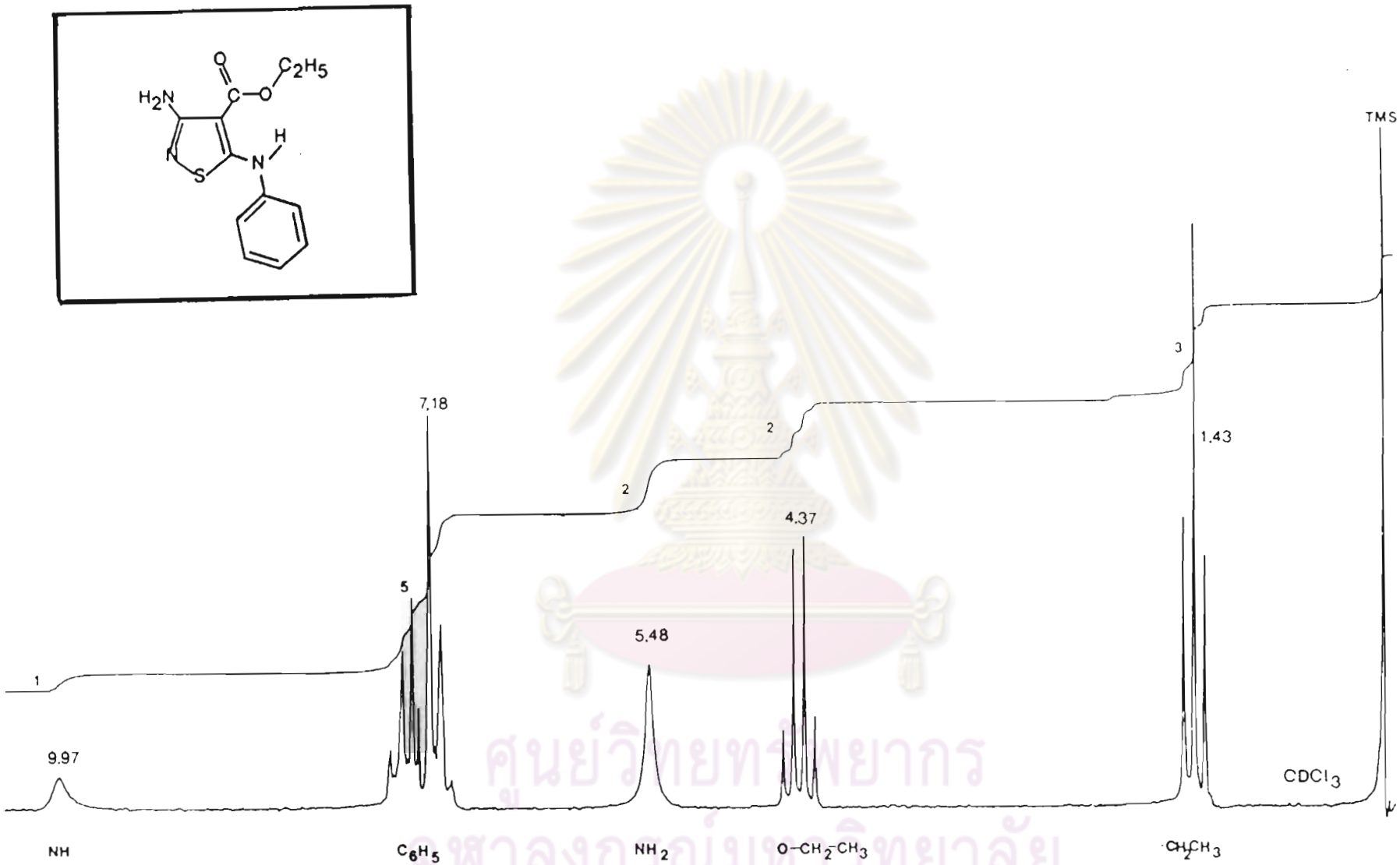


FIGURE 20: THE NMR SPECTRUM OF 3-AMINO-4-CARBETHOXY-5-PHENYLAMINOISOTHIAZOLE

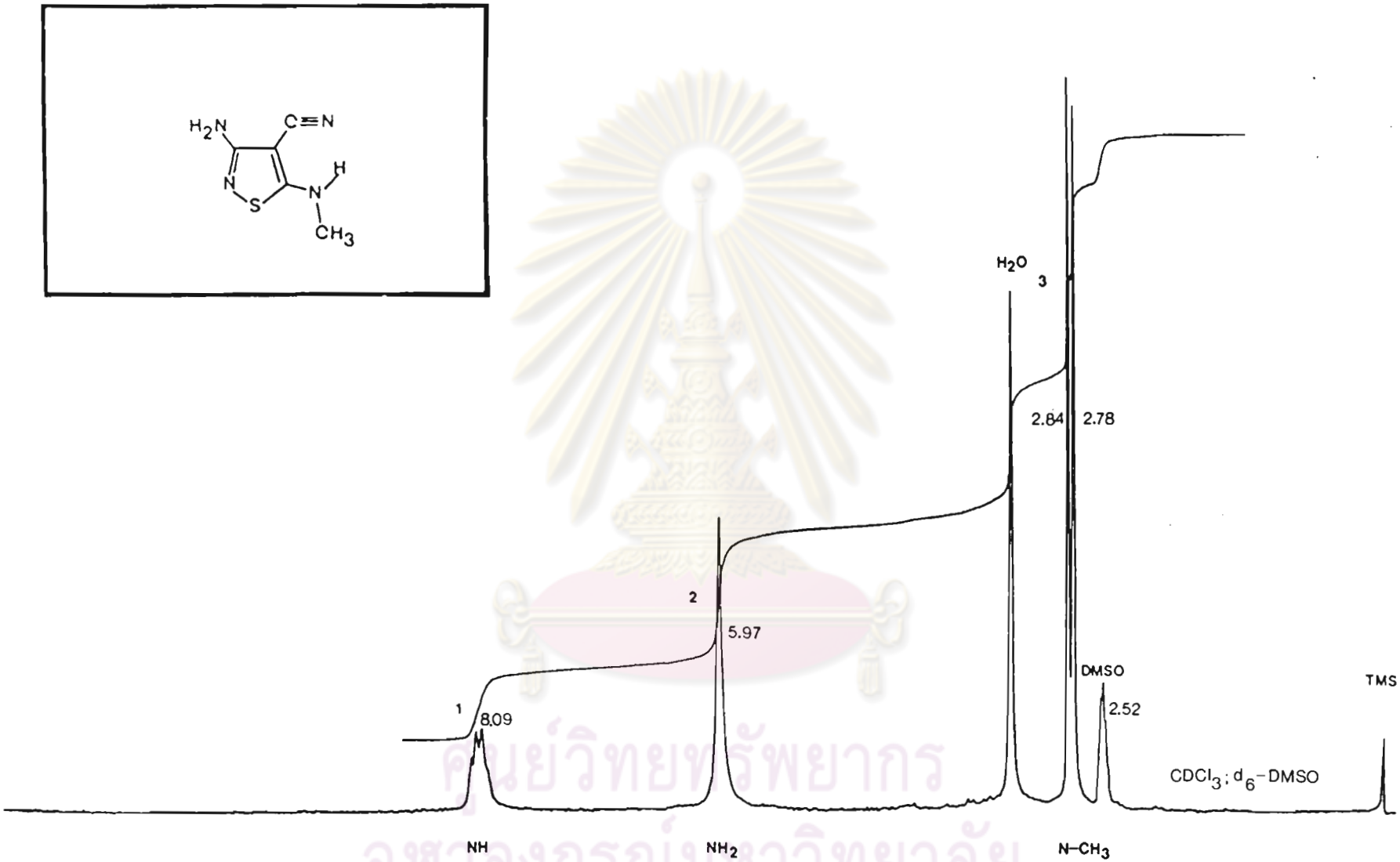


FIGURE 21: THE NMR SPECTRUM OF 3-AMINO-4-CARBETHOXY-5-METHYLAMINOISOTHIAZOLE

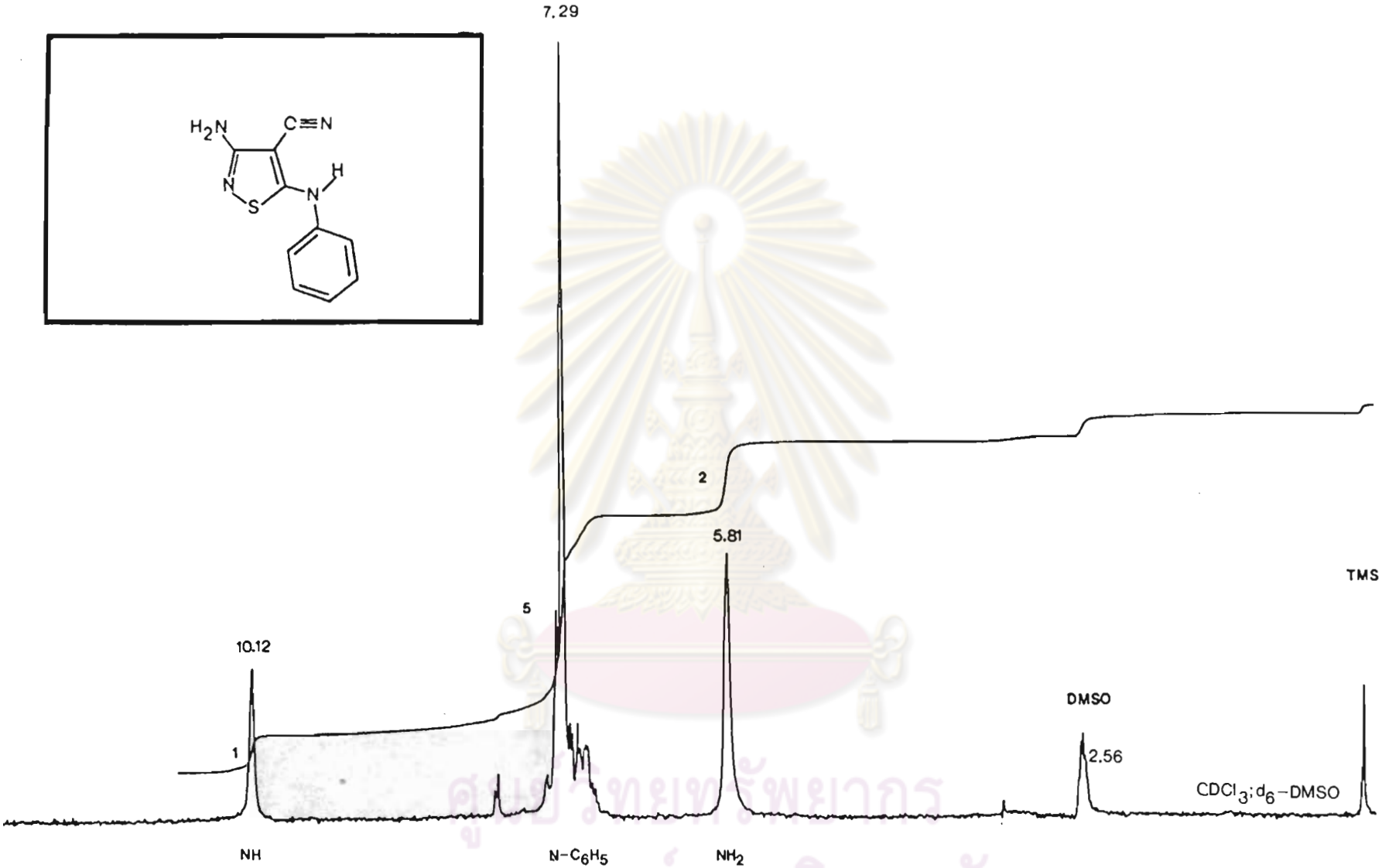


FIGURE 22: THE NMR SPECTRUM OF 3-AMINO-4-CARBETHOXY-5-PHENYLAMINOISOTHIAZOLE

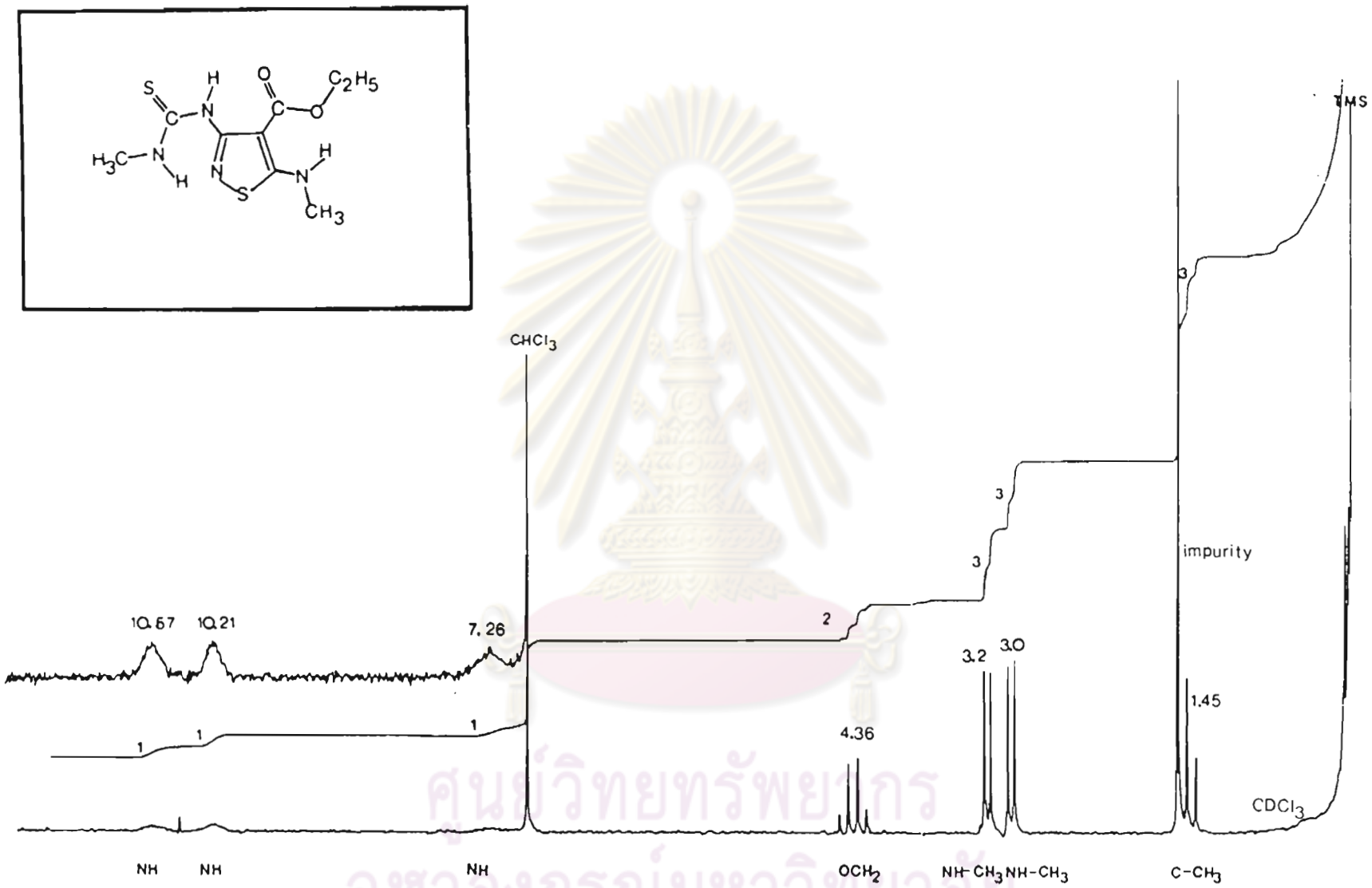


FIGURE 23: THE NMR SPECTRUM OF N-METHYL-N'-(4-CARBETHOXY-5-METHYLAMINO-3-ISOTHIAZOLYL)-THIOUREA

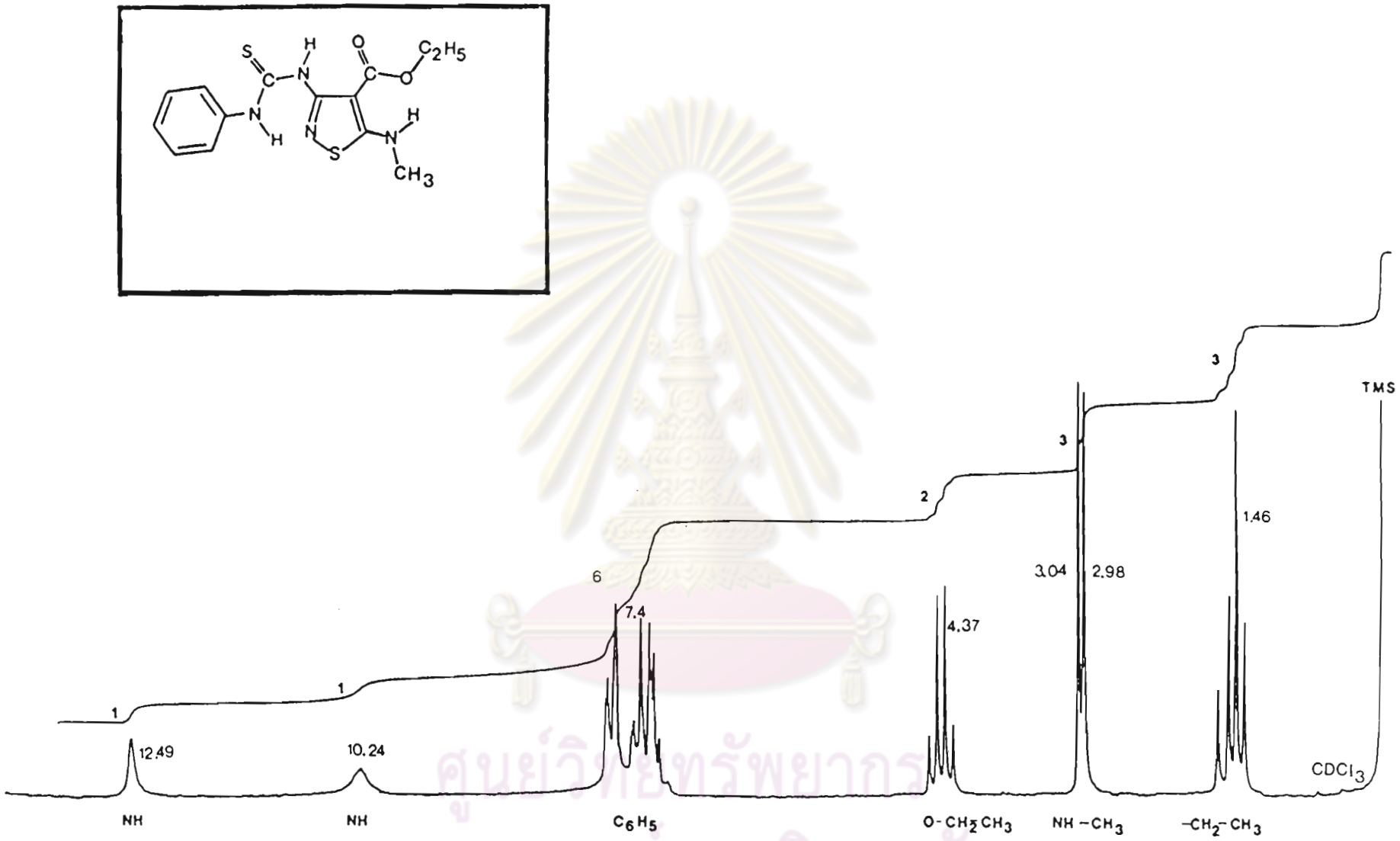


FIGURE 24: THE NMR SPECTRUM OF N-PHENYL-N'-(4-CARBETHOXY-5-METHYLAMINO-3-ISOTHIAZOLYL)-THIOUREA

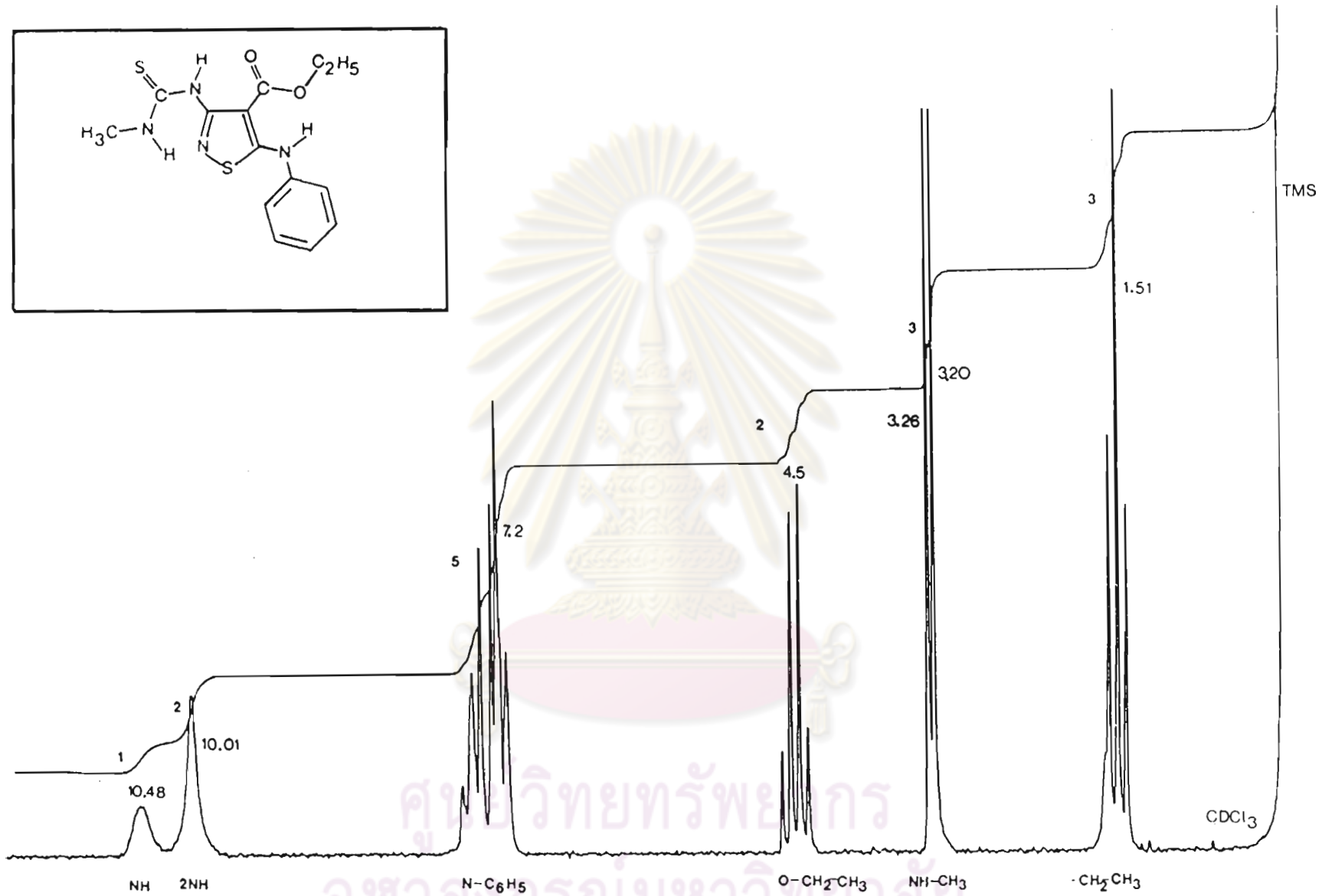


FIGURE 25: THE NMR SPECTRUM OF N-METHYL-N'-(4-CARBETHOXY-5-PHENYLAMINO-3-ISOTHIAZOLYL)-THIOUREA

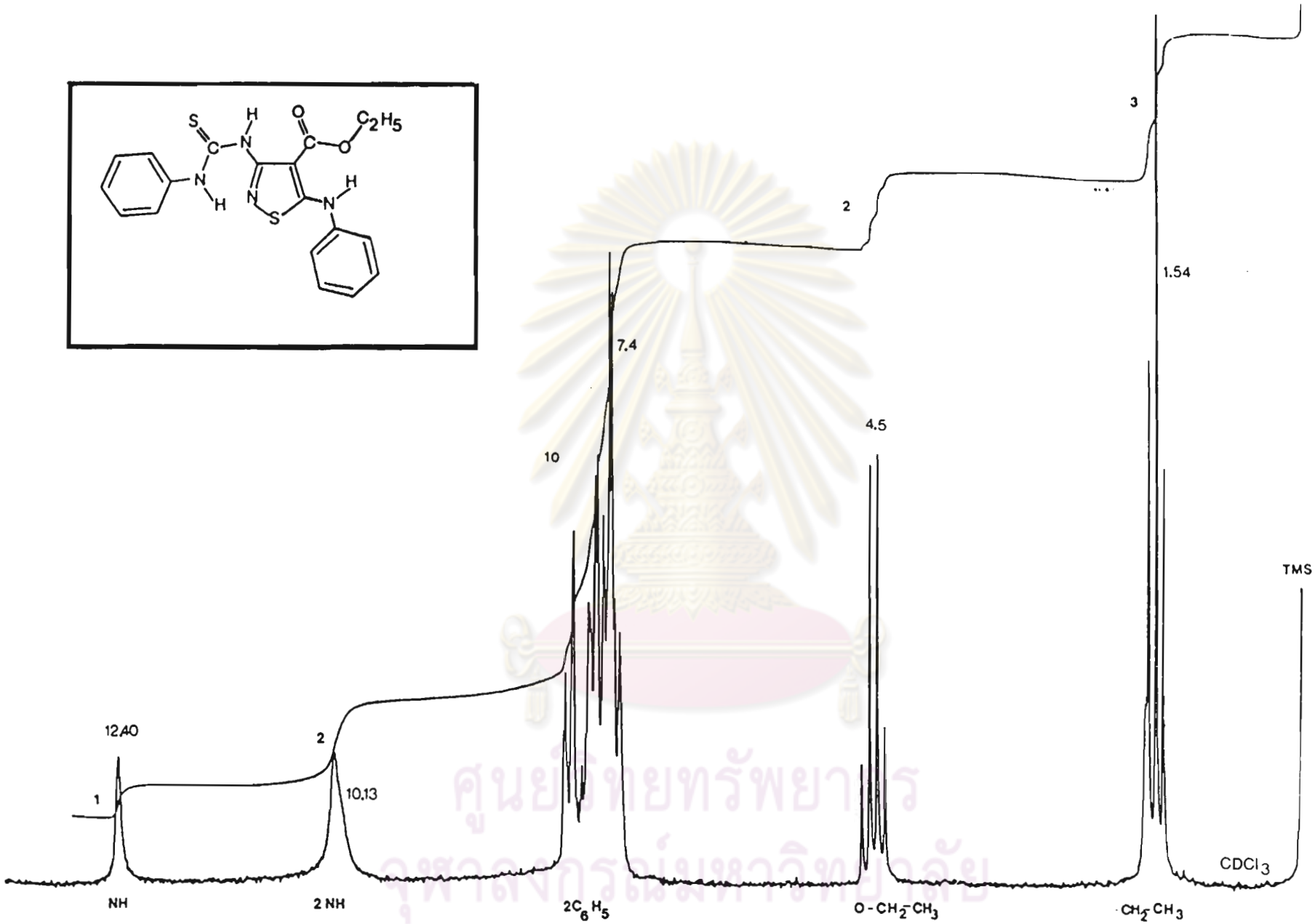


FIGURE 26: THE NMR SPECTRUM OF N-PHENYL-N'-(4-CARBETHOXY-5-PHENYLAMINO-3-ISOTHIAZOLYL)-THIOUREA

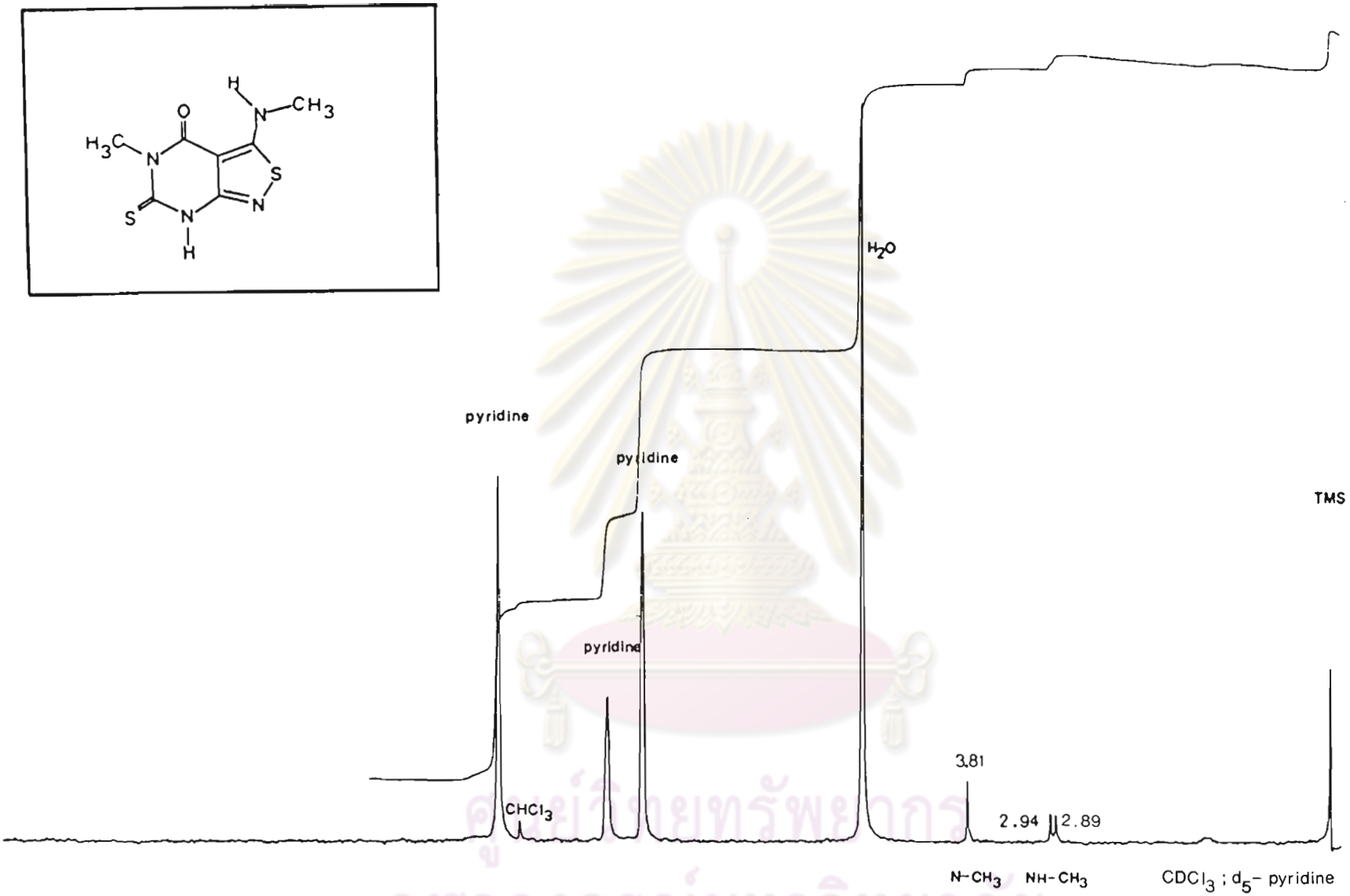


FIGURE 27: THE NMR SPECTRUM CF 5-METHYL-3-METHYLAMINOISOTHIAZOLO[3,4-d]-FYRIMIDINE-4-CNE-6(7H)-THIONE

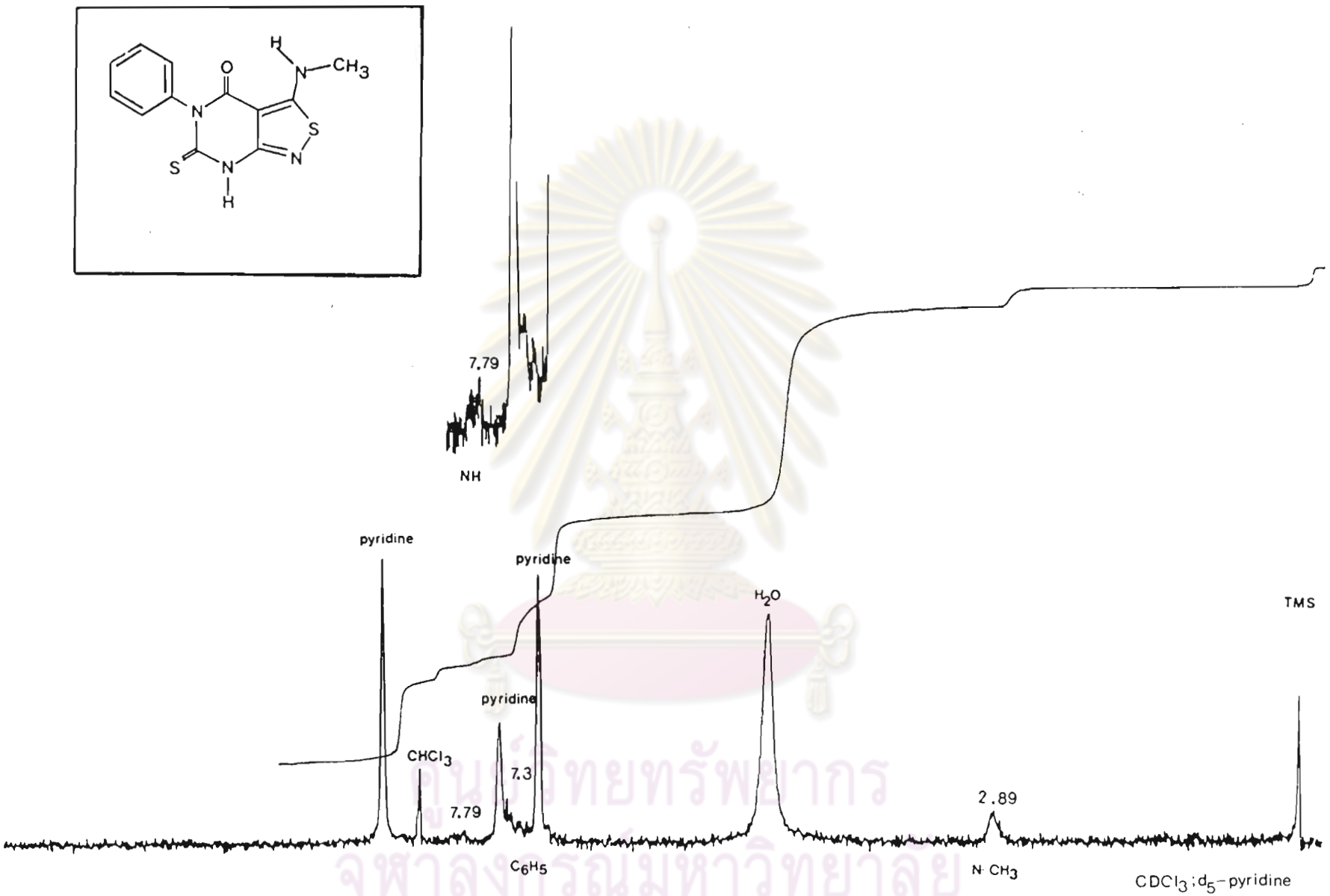


FIGURE 28: THE NMR SPECTRUM OF 5-PHENYL-3-METHYLAMINOISOTHIAZOLO[3,4-d]-PYRIMIDINE-4-ONE-6(7H)-THIONE

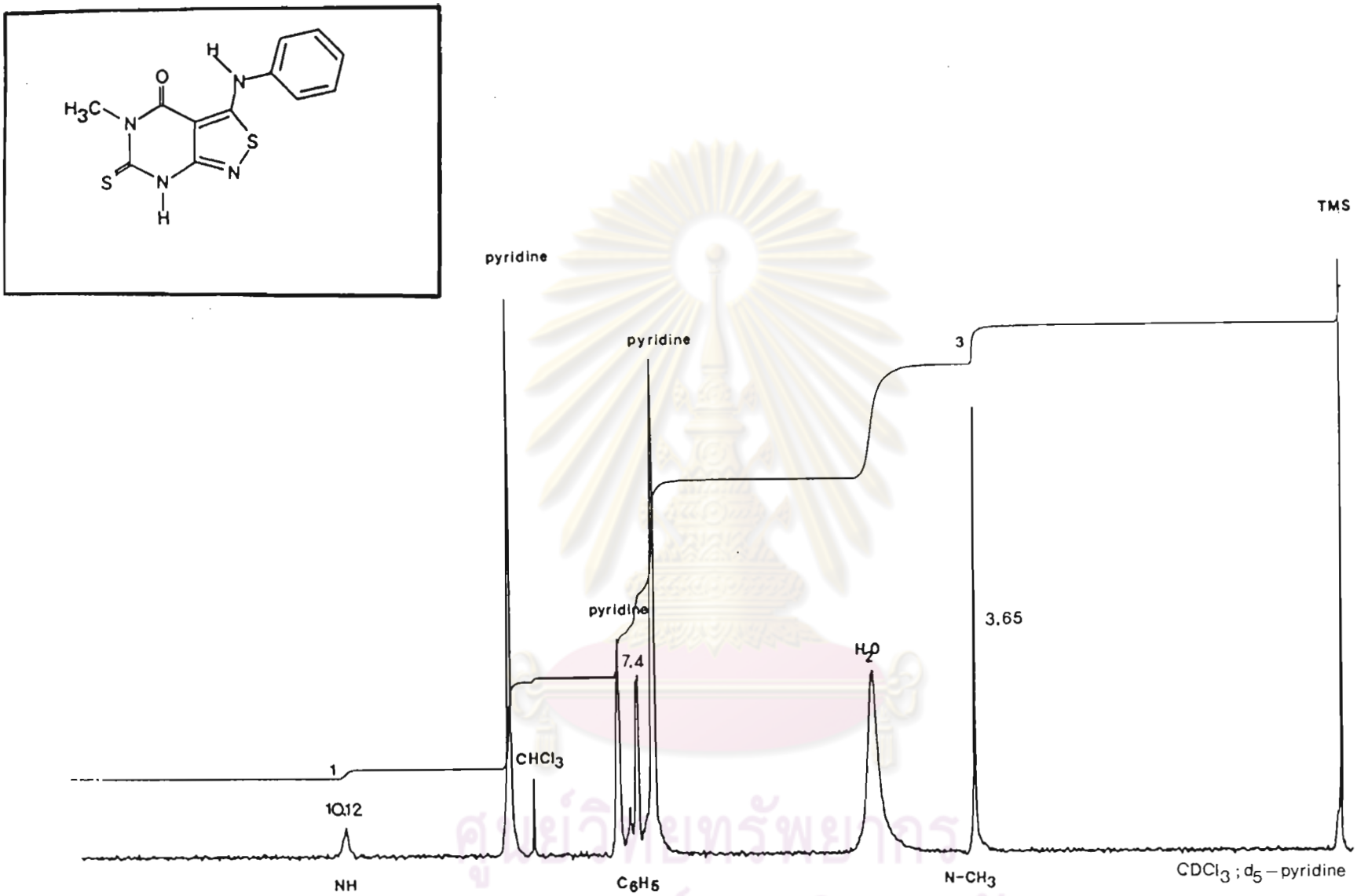


FIGURE 29: THE NMR SPECTRUM OF 5-METHYL-3-PHENYLAMINOISOTHIAZOLO[3,4-d]-PYRIMIDINE-4-ONE-6(7H)-THIONE

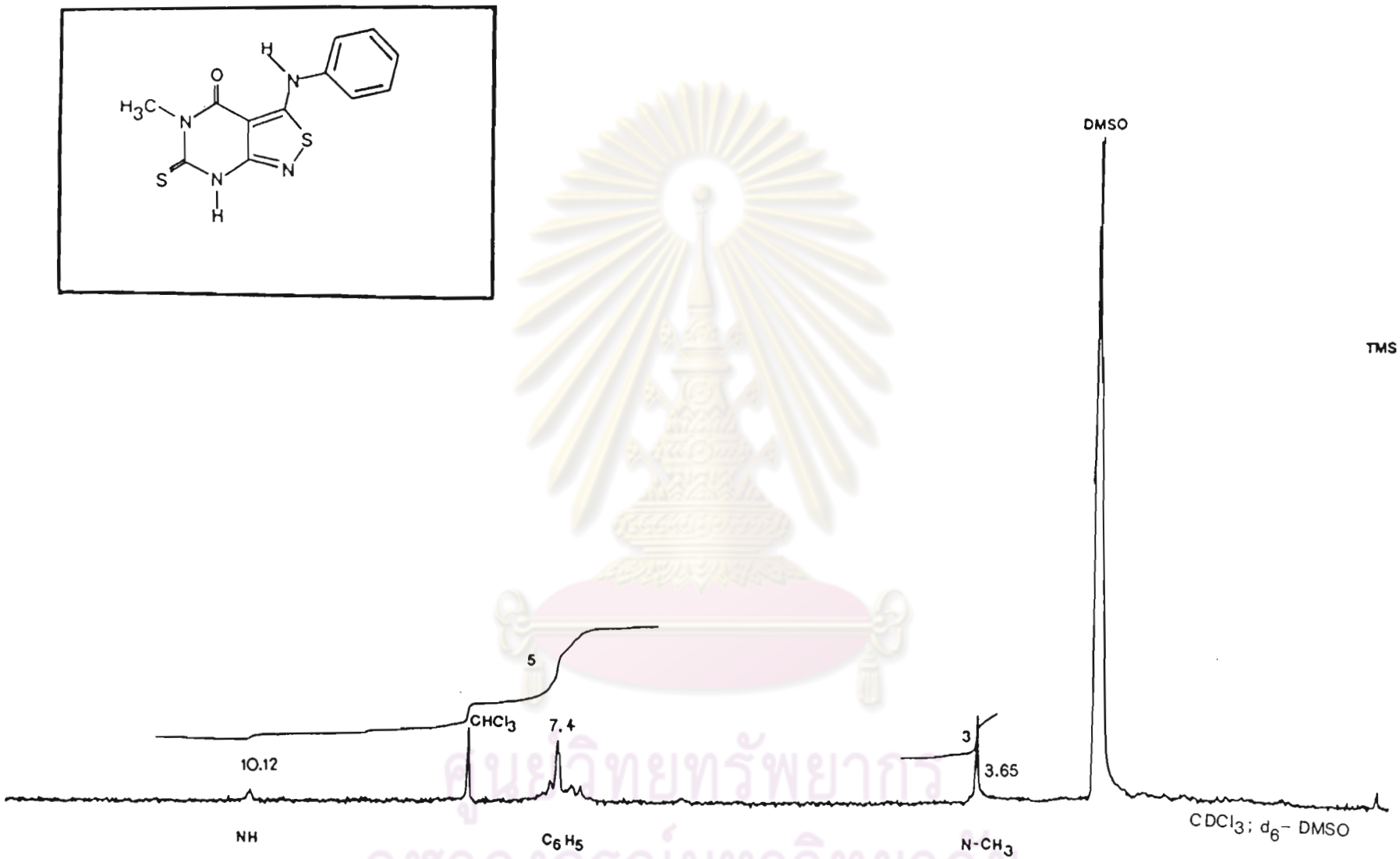


FIGURE 29: THE NMR SPECTRUM OF 5-METHYL-3-PHENYLAMINOISOTHIAZOLO[3,4-d]-PYRIMIDINE-4-ONE-6(7H)-THIONE

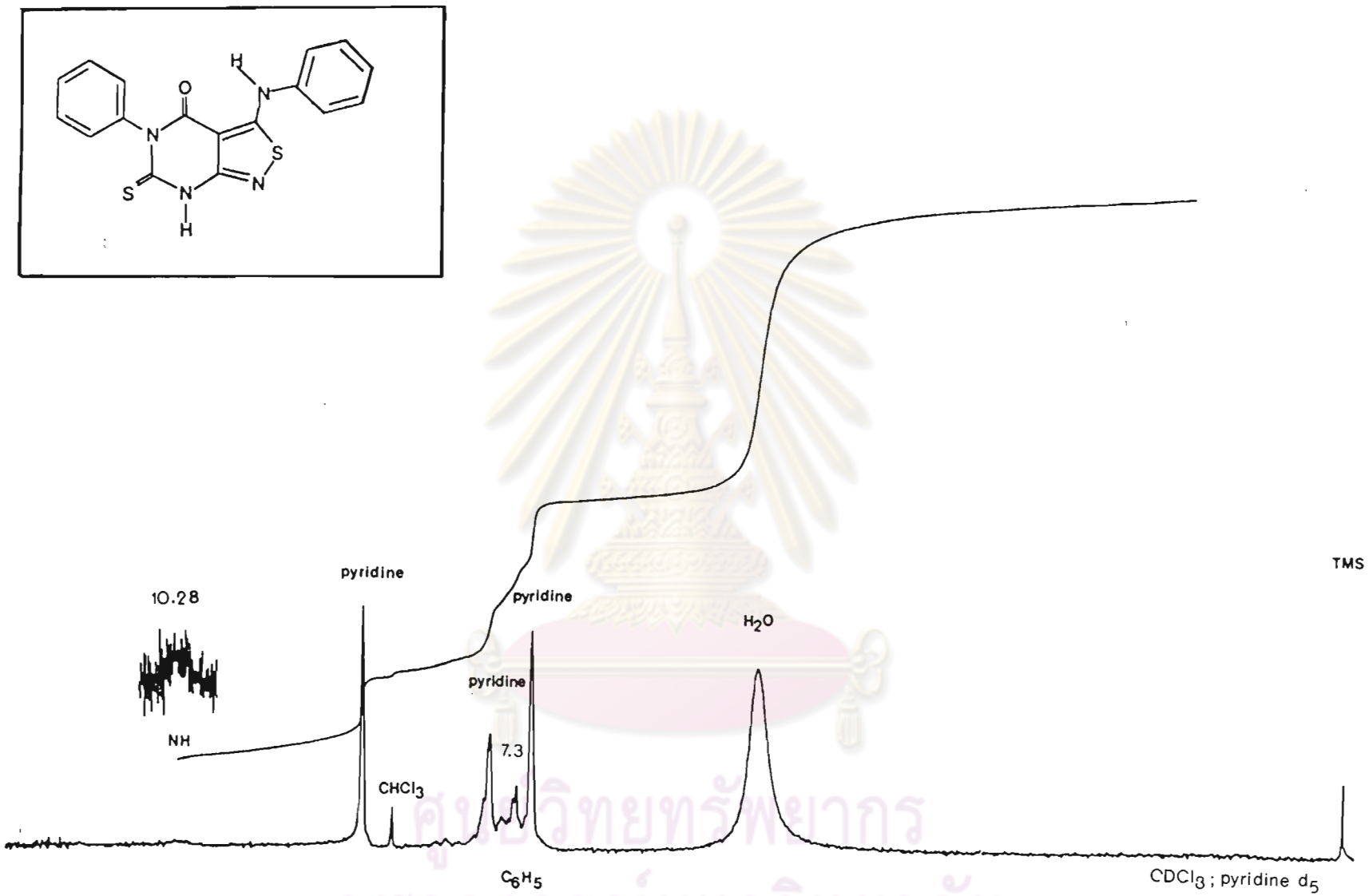


FIGURE 30: THE NMR SPECTRUM OF 5-PHENYL-3-PHENYLAMINOISOTHIAZOLO[3,4-d]-PYRIMIDINE-4-ONE-6(7H)-THIONE

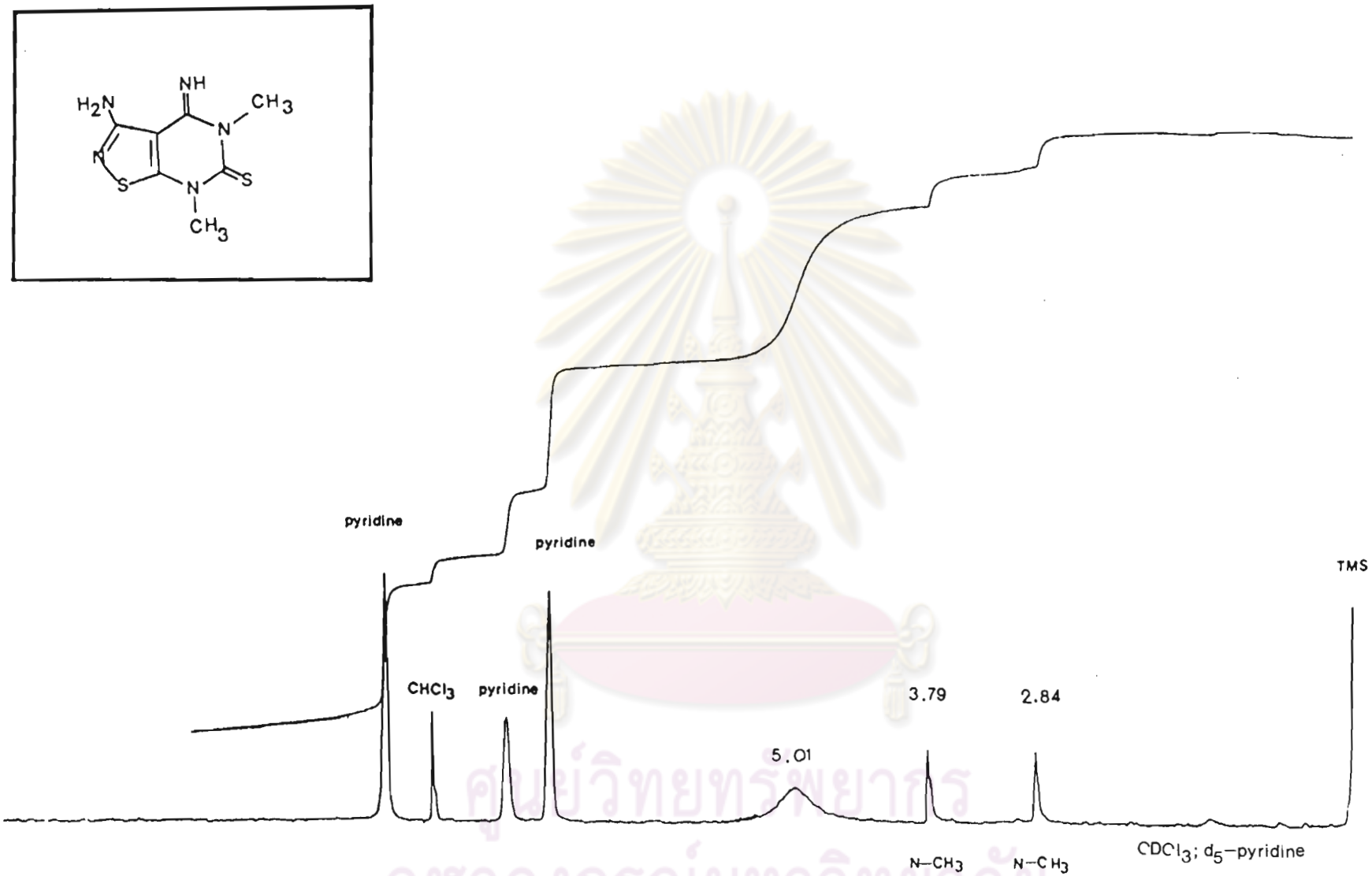


FIGURE 31: THE NMR SPECTRUM OF 5,7-DIMETHYL-3-AMINOISOTHIAZOLO[5,4-d]-PYRIMIDINE-4-IMINE-6-THIONE

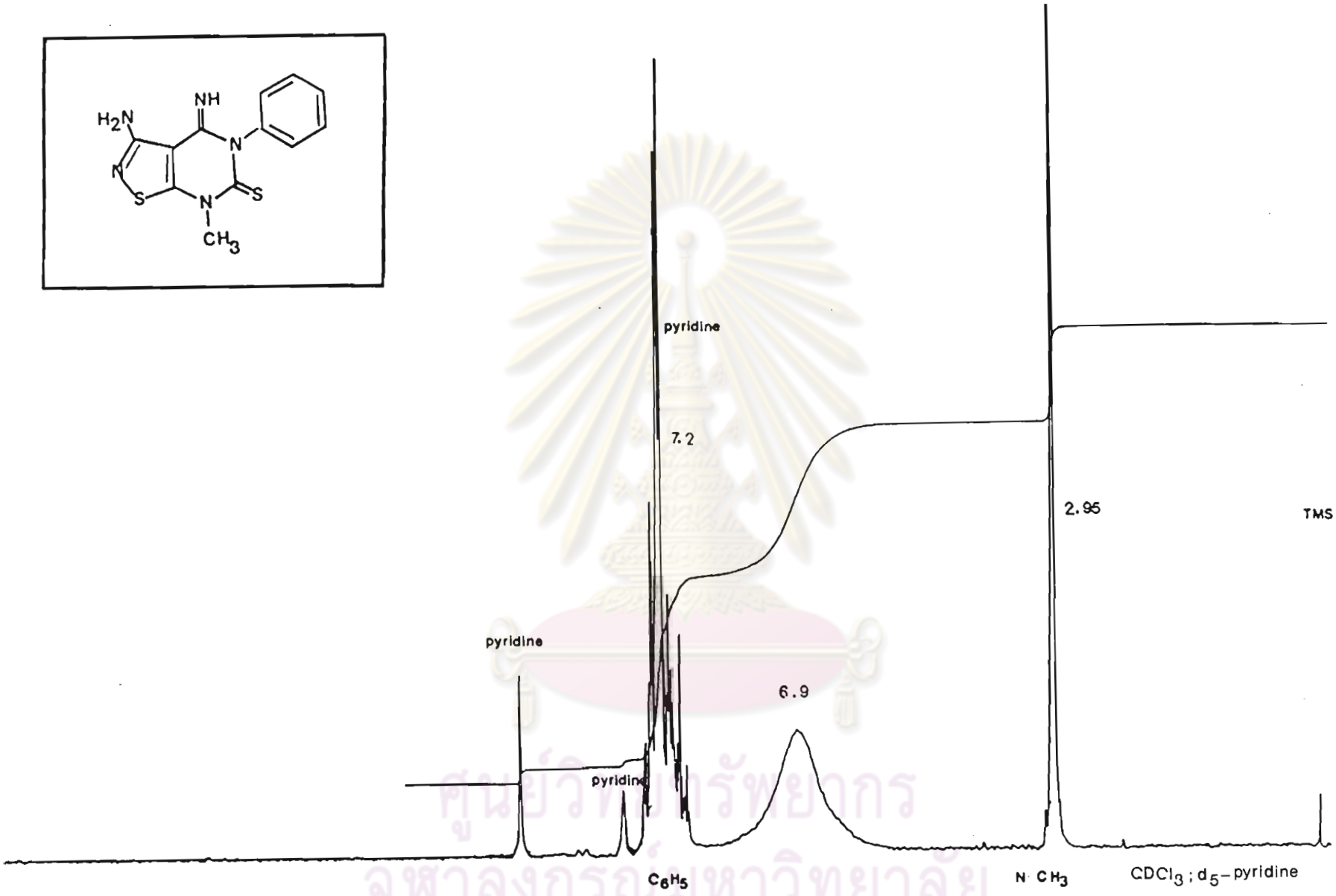


FIGURE 32: THE NMR SPECTRUM OF 5-PHENYL-7-METHYL-3-AMINOISOTHIAZOLO[5,4-d]-PYRIMIDINE-4-IMINE-6-THIONE

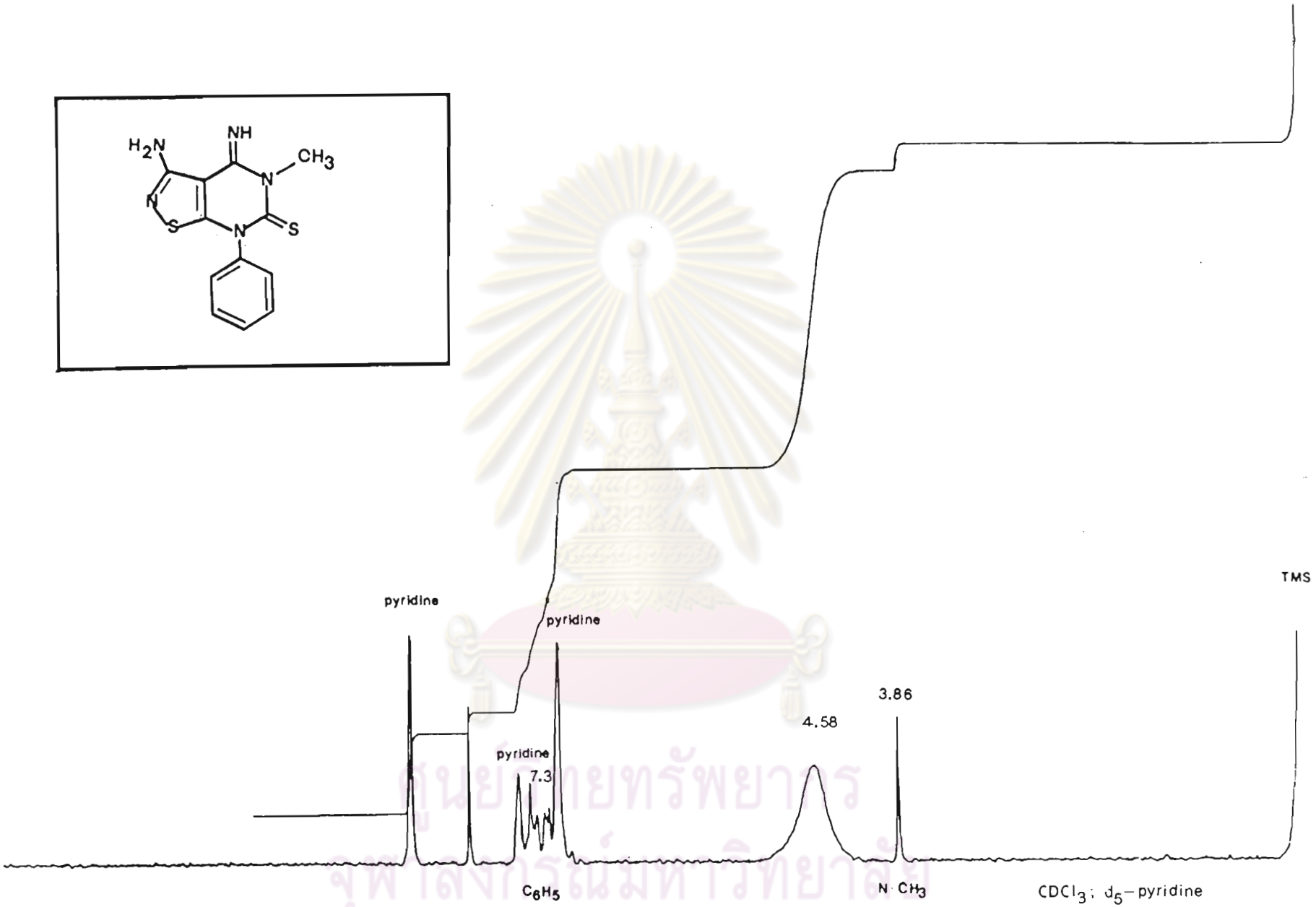


FIGURE 33: THE NMR SPECTRUM OF 5-METHYL-7-PHENYL-3-AMINOISOTHIAZOLO[5,4-d]-PYRIMIDINE-4-IMINE-6-THIONE

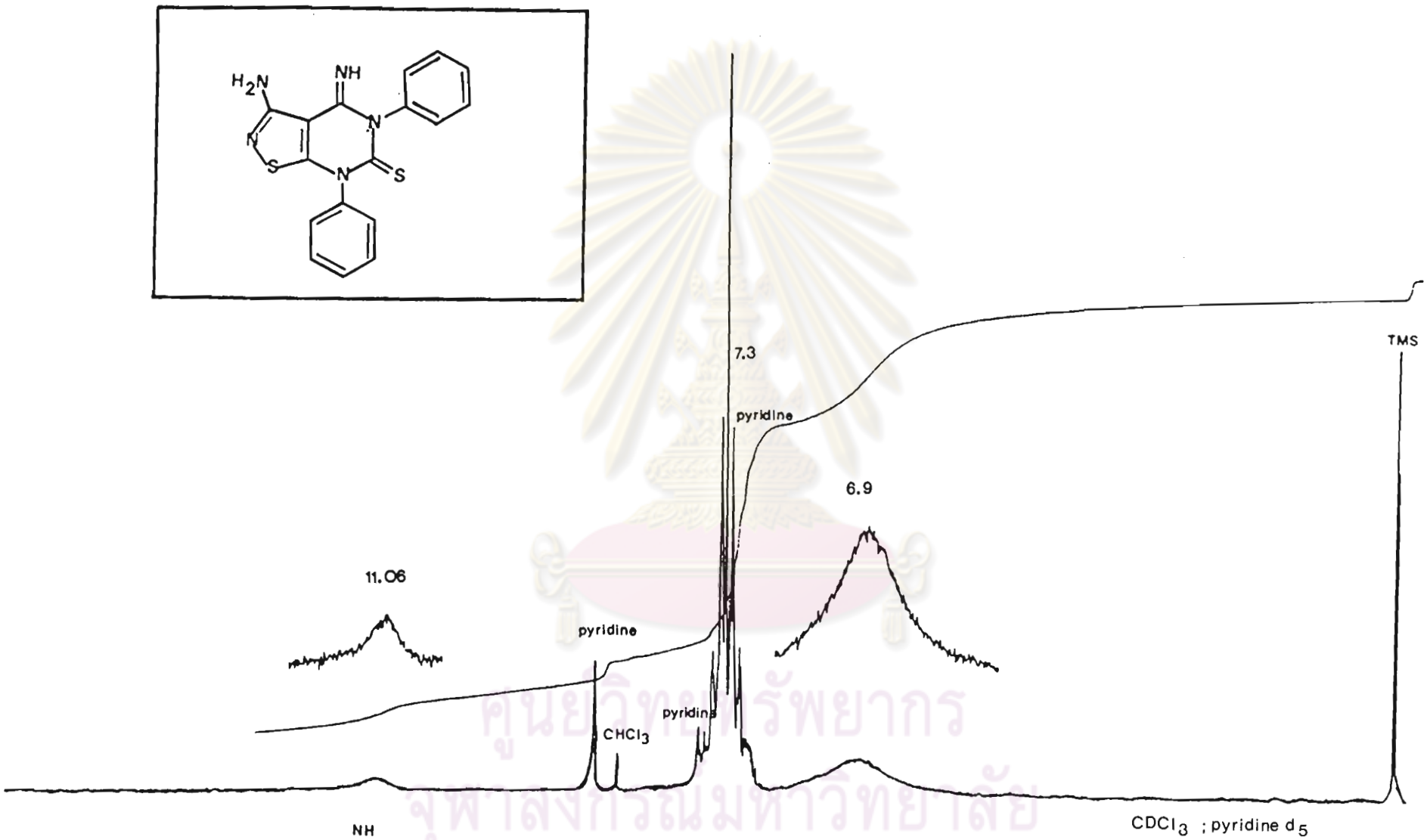


FIGURE 34: THE NMR SPECTRUM OF 5,7-DIPHENYL-3-AMINOISOTHIAZOLO[5,4-d]-PYRIMIDINE-4-IMINE-6-THIONE

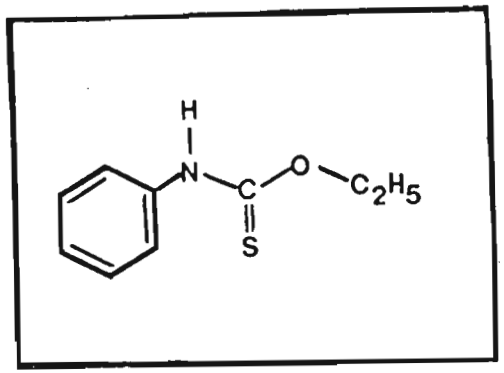


FIGURE 35: THE NMR SPECTRUM OF N-PHENYLTHIOCARBAMATE

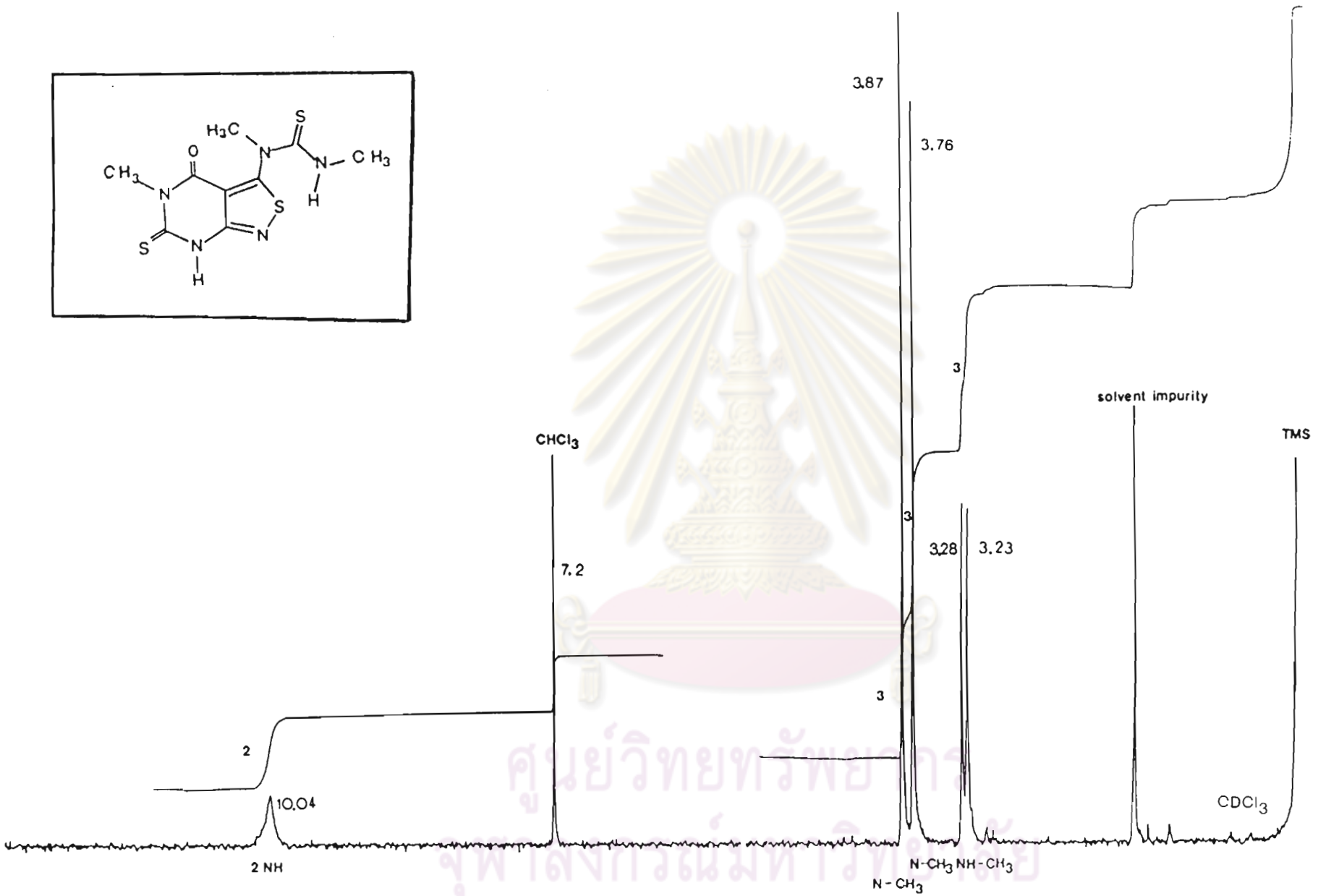


FIGURE 36: THE NMR SPECTRUM OF 3-[N-METHYL,N-(METHYLTHIOCARBAMYL)]AMINO-5-METHYL-ISOTHIAZOLO[3,4-d]PYRIMIDINE-4-ONE-6(7H)THIONE

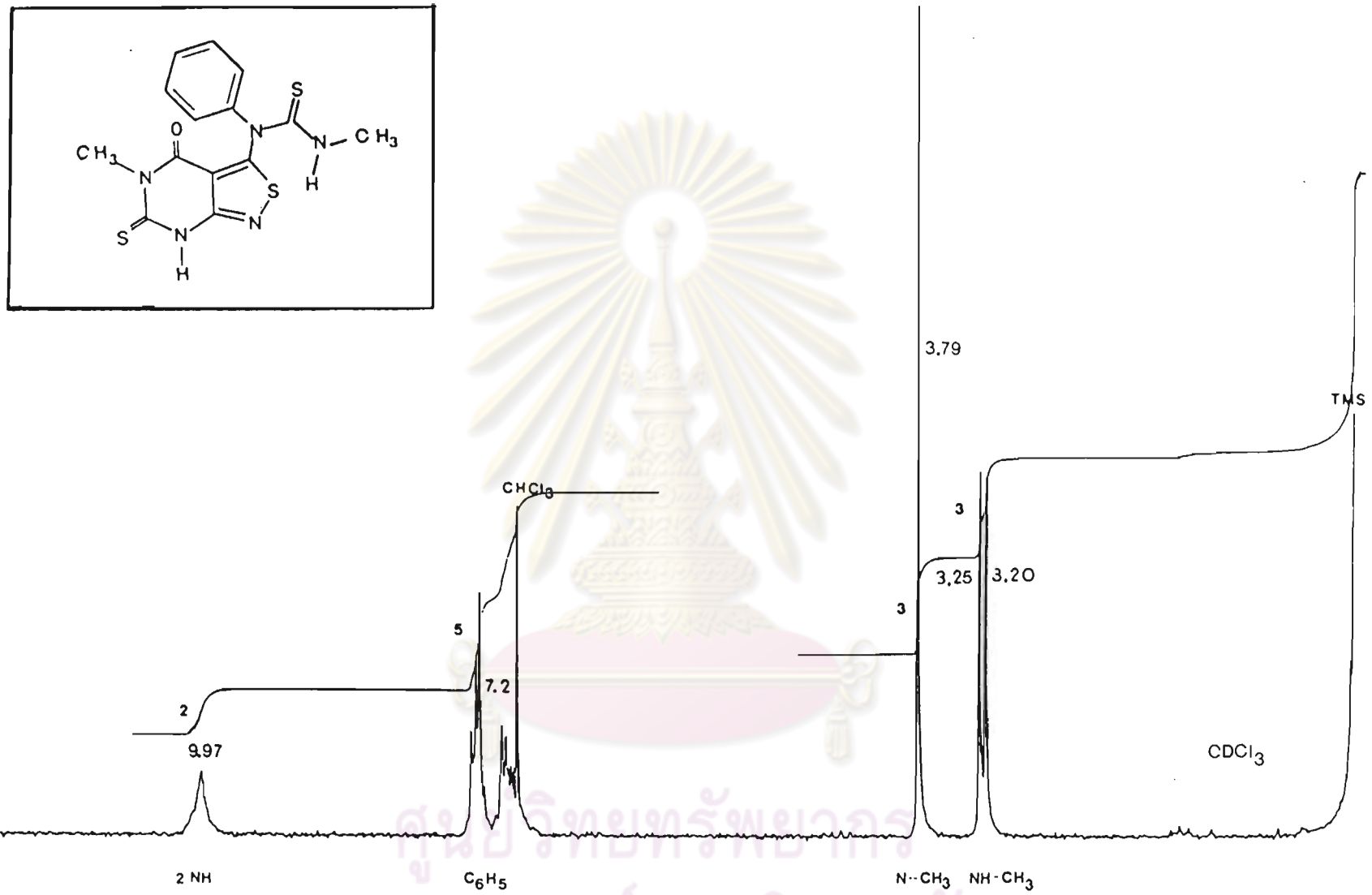


FIGURE 37: THE NMR SPECTRUM OF 3-[N-PHENYL,N-(METHYLTHIOCARBAMYL)]AMINO-5-METHYL-ISOTHIAZOLO[3,4-d]PYRIMIDINE-4-ONE-6(7H)THIONE

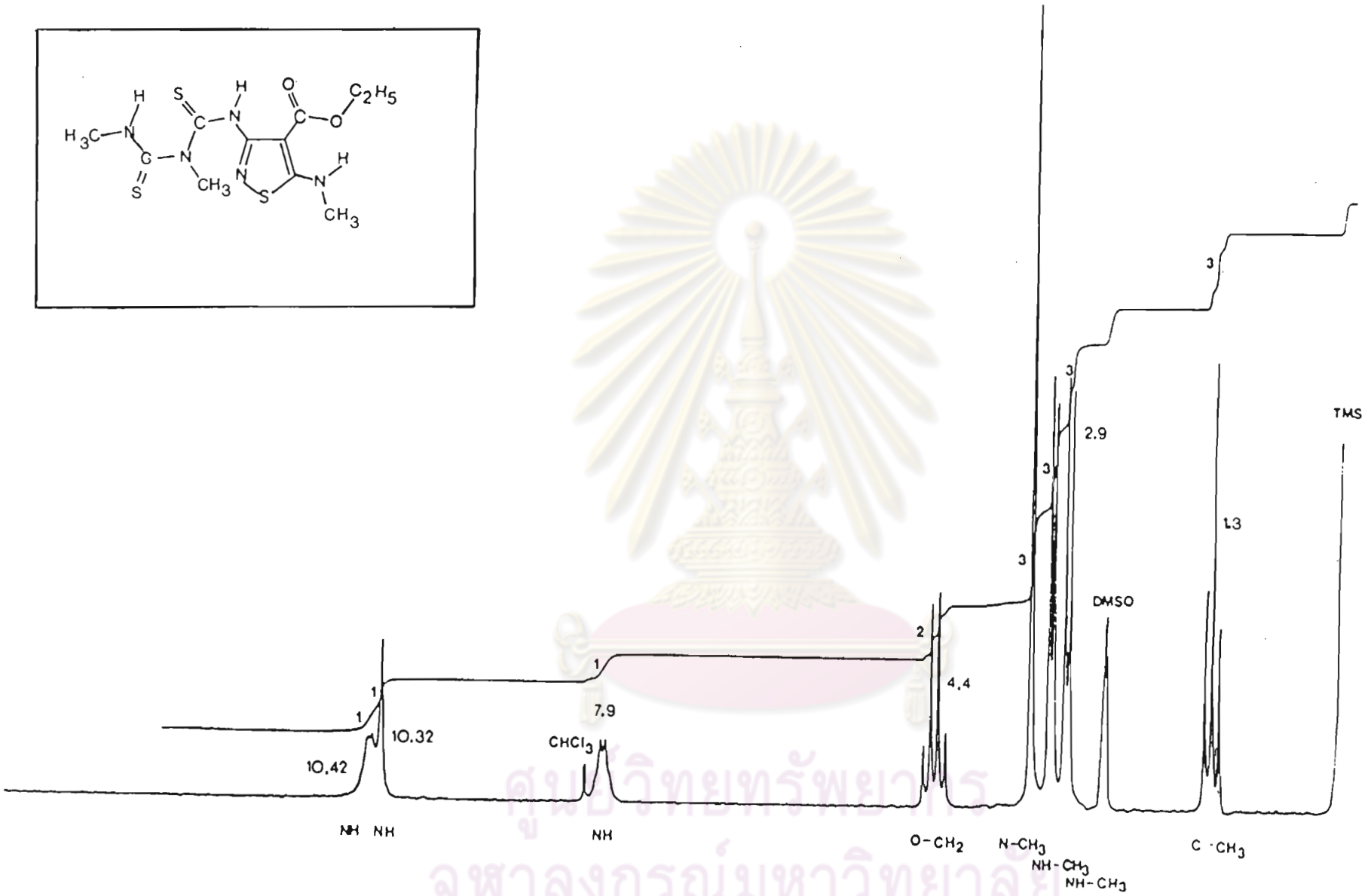


FIGURE 38: THE NMR SPECTRUM OF N-METHYL-N'-METHYL-N"-(4-CARBETHOXY-5-METHYL AMINO-3- AMINOISOTHIAZOLYL) THIOBIURET

VITA

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She started her career as a faculty member in the Department of Pharmaceutical Chemistry , Faculty of Pharmacy , Prince of Songkla University. One year later she was admitted to the Graduate School at Chulalongkorn University in Department of Pharmaceutical Chemistry.

Upon completion of her graduate study , she resumed her current position in the Department of Pharmaceutical Chemistry , Faculty of Phamacy , Prince of Songkla University , Songkla , Thailand.