การสังเคราะห์คอมบิเนทอเรียลไลบรารีของ 4,6-ไดอะมิโน-1,2-ไดไฮโดร-1,3,5-ไตรอาซีน ในวัฏภาคสารละลาย

นางสาวหนึ่งฤทัย แสแสงสีรุ้ง

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SYNTHESIS OF SOLUTION PHASE COMBINATORIA L LIBRARIES OF 4,6-DIAMINO-1,2-DIHYDRO-1,3,5-TRIAZINES

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ได้พัฒนาวิธีการที่มีประสิทธิภาพสำหรับการสังเคราะห์ของผสม คอมบิเนทอเรียลของ 1-เอริล-4,6-ไดอะมิโน-1,2-ไดไฮโดร-1,3,5-ไตรอาซีนในวัฏภาคสารละลาย วิธีการนี้มีพื้นฐานอยู่บนปฏิกิริยาการปิดวงแหวนโดยการควบแน่น มีกรดเป็นตัวเร่งปฏิกิริยาระหว่างเอริลไบกัวไนด์ไฮโดร-คลอไรด์ และ สารประกอบคาร์บอนิลชนิดต่างๆ โดยใช้ไตรเอทิลออร์โธอะซิเตตเป็นตัวขจัดน้ำ ชุดของ คอมบิเนทอเรียลไลบรารีที่ประกอบด้วยผลิตภัณฑ์ 96 ชนิด ถูกเตรียมจาก เอริลไบกัวไนด์ 6 ชนิด และสารประกอบคาร์บอนิล 16 ชนิด การสกรีนโดยวิธีอิเทอเรทีฟดีคอนโวลูชั่น แสดงให้เห็นว่าสารบางตัวมีฤทธิ์ยับยั้งเอ็มไซม์ ไดไฮโดรโฟเลตรีดักเตส ของพลาสโมเดียม ฟาลซิพารัมทั้งชนิดธรรมชาติและชนิดมิวแตนท์ (A16VS108T) ได้ดีกว่าไซโคลกัวนิล

ภาควิชา……เคมี…….. .ลายมือชื่อนิสิต……..
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An efficient method for the synthesis in solution phase of combinatorial mixtures of 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazine has been developed. This method based on an acid catalyzed cyclocondensation between arylbiguanides hydrochloride and carbonyl compounds in the presence of triethyl orthoacetate as dehydrating agent. A 96-membered combinatorial library was created from 6 aryl-biguanides and 16 carbonyl compounds. Screening of the library by iterative deconvolution method showed that some compound inhibited both wild-type and A16VS108T mutant dihydrofolate reductase enzymes of *Plasmodium falciparum* more effectively than cycloguanil.

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CONTENTS

page

Page

LIST OF FIGURES

page

Page

page

page

page

page

page

page

page

page

page

LIST OF SCHEMES

page

LIST OF TABLES

page

page

page

page

LIST OF ABBREVIATIONS

i) Nomenclature and abbreviations of enzymes, amino acids and biochemicals

DHFR	dihydrofolate reductase
DHFR-TS	dihydrofolate reductase-thymidylate synthase
DNA	deoxyribonucleic acids
FH_4	tetrahydrofolate
GTP	guanosine triphosphate
pfDHFR	*Plasmodium falciparum* dihydrofolate reductase
A	Alanine
S	Serine
T	Threonine
V	Valine

ii) Miscellaneous

abs.	absolute
°C	degree celcius
Calc.	calculated
$CDCl_3$	deuterated chloroform
conc.	concentrated
Cyc	cycloguanil
d	doublet
dd	doublet of doublet
DMSO-*d6*	deuterated dimethyl sulfoxide
D_2O	deuterium oxide
eq	equivalents
ESI	electrospray ionization
Et_2O	diethyl ether
g	gram
Hz	hertz
hr (s)	hour (s)

J	coupling constant
K_i	inhibition constant
m	multiplet
MALDI-TOF	matrix-assisted laser desorption/ionization-time of flight
MHz	megahertz
mg	milligram
min	minute
mL	milliliter
MIBK	methyl isobutyl ketone
mmol	millimole
mut.	Mutant
m/z	mass per charge ratio
nM	nanomolar
NMR	muclear magnetic resonance
ppm	part per million
Pyr	pyrimethamine
q	quartet
s	singlet
t	triplet
TEOA	triethyl orthoacetate
TLC	thin layer chromatography
t_R	retention time
ν_{max}	the weavelength at maximum absorption
wt.	wild-type
δ	chemical shift

CHAPTER I

INTRODUCTION

1.1 Literature reviews

1.1.1 Malaria

Although the annihilation of malaria has been one of human's continuous effort for a long time, the disease remains a major health threat in many areas of the world, especially in tropical and subtropical countries including Africa and South-East Asia.[1] The disease infects 300 to 500 million people each year. The World Health Organization (WHO) estimates that between 1.5 and 2.7 million people die from malaria every year, either directly or in association with acute respiratory infections and anemia, and up to 1 million of those deaths are among children younger than five years old. Moreover, more people die from malaria each year than have died from AIDS in the last seventeen years.[2]

The cause of malaria in human is one of the four species of protozoan parasites of the *Plasmodium* genus; *Plasmodium falciparum, P. vivax, P. ovale, P.malariae,* each of which presents slightly different clinical symptoms. *P. falciparum* is the most geographically widespread of the four and the most dangerous, causing the majority of malaria-related morbidity and mortality.

A female anopheles mosquito, which is present in almost all countries in the tropics and subtropics, transmits parasites from one person to another. The parasite is carried by the blood to the victim's liver where they grow and increase in number of cells. After 9-16 days, they again multiply and begin destroying the red blood cell. The symptoms of malaria illness include headache, back pain, chills, muscle ache, increased sweating, malaise, nausea and sometime vomiting, diarrhea, and cough. Of all the four species of malarial parasite, only falciparum malaria can progress rapidly to obstruct the blood vessels in the brain known as the cerebral stage. Untreated cases can progress to coma, renal failure, liver failure, convulsions and eventually death.

The plasmodium life cycle

The life cycle of the parasite in both mosquitoes and human is complex (**Figure 1.1**) as is the terminology of the various parasite development stages.[3] When an infected mosquito bites the human, sporozoites are injected into the blood stream of the human victim and travel to liver tissue where they invade parenchymal cell. During development and multiplication in the liver known as preerythrocytic stage, there is no sign of infection. After a period of time, merozoites (5,000 - 4,000 per sporozoite) are released from the liver and the parasites take up residence in the red blood cell (erythrocytic stage). Invasion of the red cell by a merozoite results in the development of the trophozoite stage. The parasite feed upon the protein portion of hemoglobin and hemozoin, a waste product, accumulates in the host cell cytoplasm. After the parasite undergoes nuclear division, the red blood cell bursts and merozoites, waste and cell debris are released and symptoms of malaria appeared. The merozoites released and by the red cell rupture go on to infect more erythocytes. Time intervals between cell rupture (fever), infection of other erythrocytes, and then their rupture (new bouts of fever) are characteristic of the parasite species. A few merozoites become differentiated into male and female gametocytes, forms that are dormant in humans. When a mosquito takes a blood meal from the infected human, the gametocytes begin sexual reproduction in the digestive track of the mosquito. Ultimately, sporozoites from and then reside in the mosquito saliva, ready for a new cycle of infection.

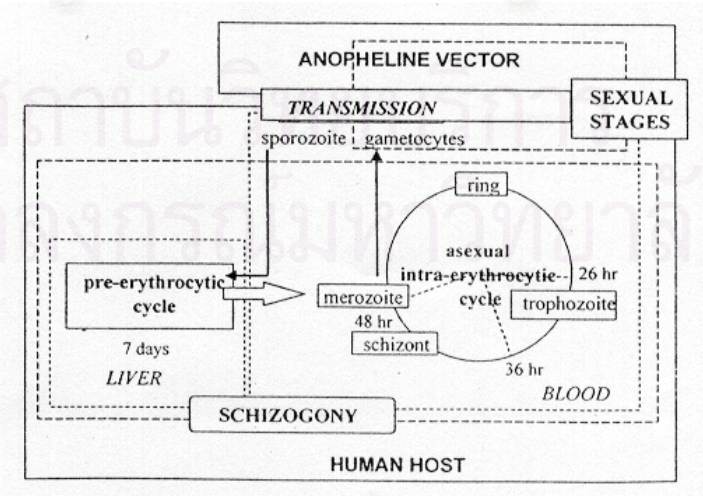


Figure 1.1: Plasmodia life cycle

Drugs used in the treatment of malaria

Malaria can normally be treated by anti-malarial drugs[4] such as quinine (**a**), chloroquine (**b**), mefloquine (**c**), sulpha (**d**), trimethoprim (**e**), pyrimethamine (**f**) and cycloguanil (**g**), artemisinin (**h**) etc. (**Figure 1.2**)

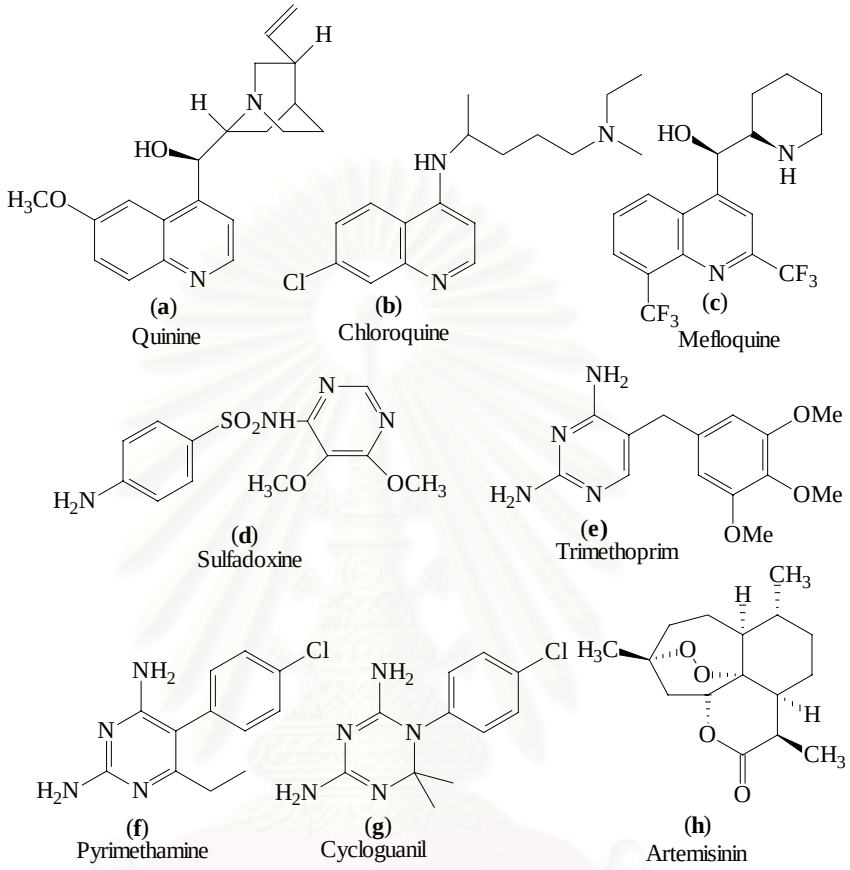
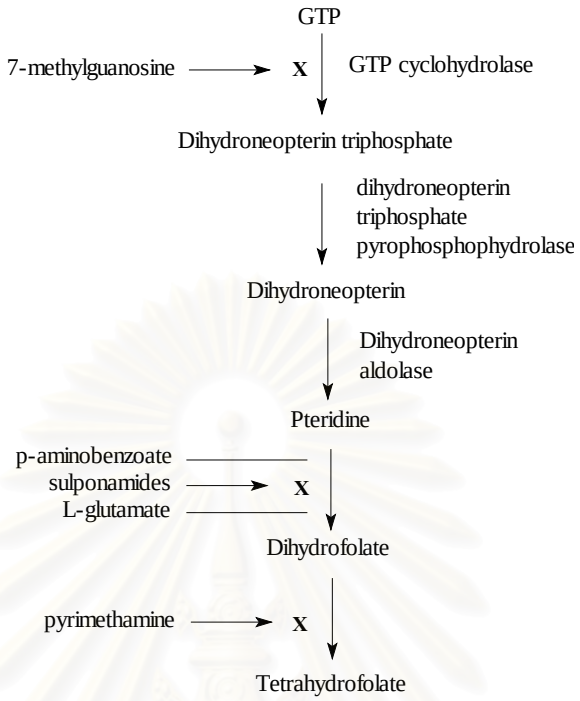


Figure 1.2: Structure of anti-malarial drugs

Using these drugs for treatment of malarial in each particular case depend on several factors such as the parasite species causing the infection, the susceptibility of the parasite strain to anti-malarial agents, the facilities and resources available for health care.

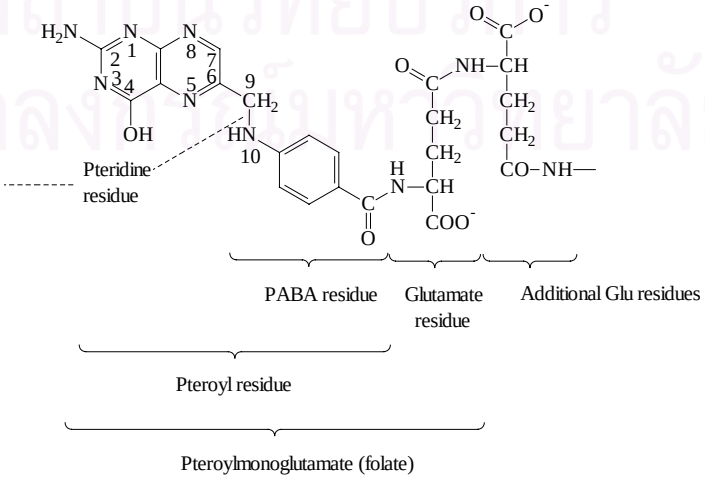
Although there are many anti-malarial drugs available, it is difficult to find only single effective drug because malaria parasites could develop resistance to anti-malarial drugs after long-term usage. In addition, life cycle of malaria parasite is similar to that of human. Many compounds were toxic to both the parasites and human. As result, searching for new anti-malarial drugs is very important. Many anti-

malarial drugs work by interfering with the folate pathway (**Scheme 1.1**) which involve the synthesis of tetrahydrofolate of malaria



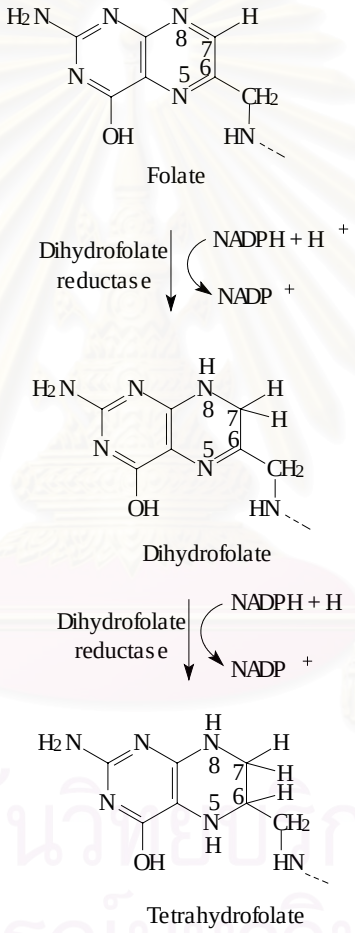
Scheme 1.1: Folate pathway in malaria disease

Folate is an essential compound for the living of malaria parasites, human and animals[5]. It is found in vegetables and green plants. Folate joined two metabolic cycles sharing a common step. One is the methionine synthesis cycle and the other is the thymidylate synthesis cycle. Methionine is an important component of protein while thymidylate is a starting material for DNA synthesis. The molecule of folate consisted of three parts, namely heterobicyclic pteridine, *p*-aminobenzoic acid and glutamic acid (**Scheme 1.2**).



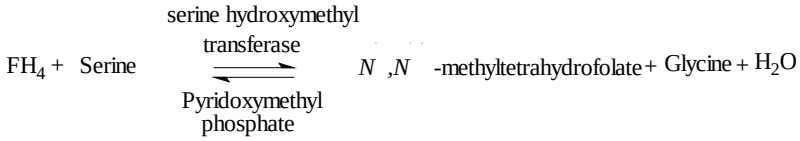
Scheme 1.2: Structure of folate

While human can not synthesize folate by themselves, malaria can do this by using enzymes. Moreover, malarial parasites could bring folate from sources outside the cells. Falciparum malaria can change guanosine triphosphate (GTP) to tetrahydrofolate (FH_4) by using GTP cyclohydrolase to change GTP to dihydroneopterin, an intermediate compound of folate synthesis (**Scheme 1.1**). Malarial parasites can change folate to tetrahydrofolate (FH_4) by a two step reduction (**Scheme 1.3**) mediated by dihydrofolate reductase (DHFR) in the presence of NADPH as co-factor. The tetrahydrofolate is used as an essential precursor of thymidylate synthesis.



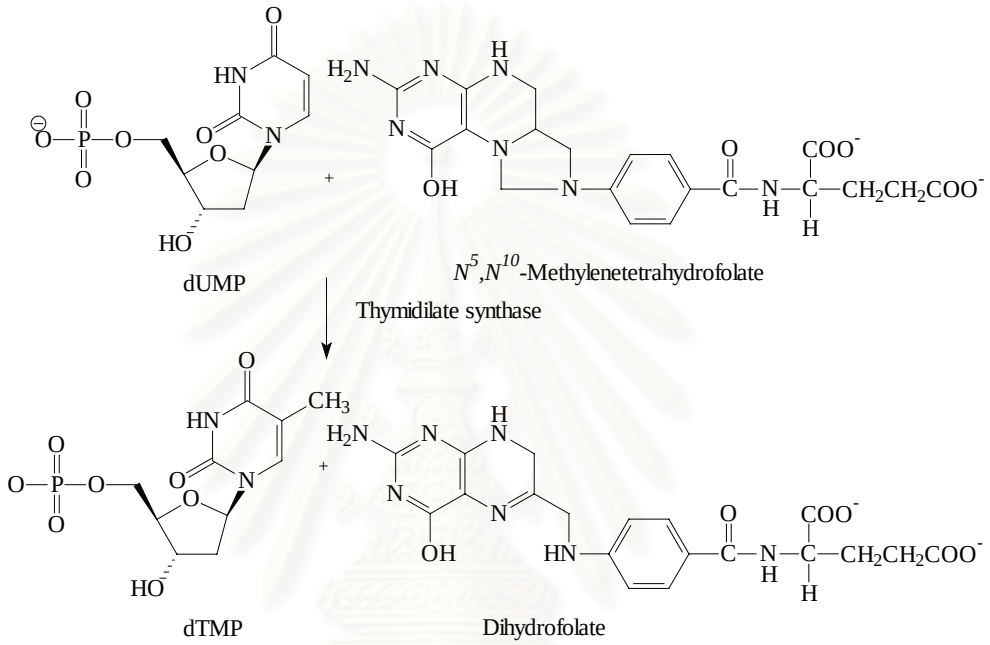
Scheme 1.3: Reduction of folate to tetrahydrofolate

Tetrahydrofolate formed from the folate pathway reacts with serine, an amino acid, to generate N^5,N^{10}-methylenetetrahydrofolate[6] as shown in **Scheme 1.4**.



Scheme 1.4: Reaction of tetrahydrofolate and serine

N^5,N^{10}-Methylenetetrahydrofolate is used in synthesis of dTMP by transfering a methylene group to dUMP as shown in **Scheme 1.5**.



Scheme 1.5: Thymidylate synthesis

Therefore, if synthesis of tetrahydrofolate was stop, the thymidylate synthesis process should be inhibited and then this could inhibit malaria disease. Production of tetrahydrofolate could be inhibited by using drugs which inhibit the dihydrofolate reductase (DHFR) domain of *P. falciparum* bifunctional enzyme dihydrofolate reductase-thymidylate synthase (DHFR-TS).[7,8] Example of these drugs include trimethoprim[4], pyrimethamine (Pyr)[9,10] and cycloguanil (Cyc),[10-12] known as "antifolates". However, at present time it was found that malaria could develop resistance to the drugs by mutation of the dihydrofolate reductase enzyme.[13] For example when the 16th position of pfDHFR was mutated from alanine to valine and the 18th position was mutated from serine to threonine, the resulting A16VS108T mutant pfDHFR became resistant to cycloguanil but not to pyrimethamine (**Table 1.1**).

Table 1.1: Inhibition Constants (K_i) of Pyr and Cyc against the Wild-Type and A16VS108T pfDHFRs

Compound	K_i (wt)[a] (nM)	K_i (mut.)[b] (nM)	K_i (mut.)/ K_i (wt)
Pyr	1.5 ± 0.2[c]	3.6 ± 0.3[c]	2.4
Cyc	1.5 ± 0.3[c]	$1{,}314 \pm 16$[c]	876

[a]Wild-type. [b]A16VS108T mutant pfDHFR. [c]Data from ref 14.

The data show that the inhibition of A16VS108T pfDHFR by Cyc was dramatically decreased compared to Pyr. So it is a challenge to find derivative of Cyc which can still effectively inhibit both the A16VS108T mutant and the wild-type enzymes. Molecular modelling suggested that the position N-3, C-4 (NH_2), N-5 and C-6 (NH_2) are important to the binding with the enzyme,[15] hence only N-1 and C-2 substituents may be varied. Analogues of Cyc (**Figure 1.3**) must be therefore different from Cyc by pattern of N-1 and C-2 substitution. The N-1 position would be changed from 4-chlorophenyl to the other substituted phenyl ring such as 4-H, 4-Me, 4-Et and 3,4-Cl_2, and at C-2 position would be changed from 2,2-dimethyl to other groups such as H, alkyl or aryl groups in order to find a better inhibitor. However, synthesis of analogues of Cyc using traditional synthesis and screening method, e.g., one compound by one reaction would be a very hard work and money and time-consuming. To make synthesis analogues of Cyc easier as well as to save cost and time, we have used combinatorial chemistry in this research.

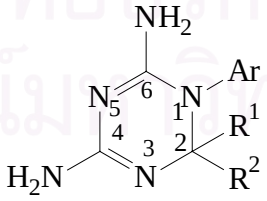


Figure 1.3: Structure of lead compound

1.1.2 Combinatorial chemistry

Traditional drug discovery depends on both serendipity and hard work. Most drugs have been found by screening naturally occurring compounds like microbial

fermentation broth, extracts from plants, fungi, algae and other marine organism, or by screening the vast collection of compounds which come from traditional chemical synthesis. The initially obtained lead compound must be further developed with trial and error to obtain a compound with higher activity that could be made into drug.

Later, there were more understanding in mechanism of biological molecule interactions such as enzyme-substrate-inhibitor complex and receptor-ligand interaction. X-ray crystallography and NMR spectroscopy technique was advanced enough to analyze 3-dimensional structure of biological molecules. Moreover, powerful modern computer has made molecular modeling more accurate and less time-consuming. Combining these knowledge together, drugs design became more rational than it was in the past. However, time-consuming synthesis and biological screening remain one of the bottle-neck in drug discovery processes. Recently, combinatorial chemistry, a new field has emerged with the aim to resolve such problem by making and screening as many compounds as possible without worrying much about the structure-activity relationship. It is sometime also referred to as "irrational drug design" .[16]

1.1.2 Combinatorial Synthesis

For over a hundred years the main task of the synthetic chemist has been the directed synthesis of one specific product which was synthesized from one substrate in one reaction vessel at a time. On the other hand, combinatorial chemistry has focused on technologies aiming to make large numbers of product which may all be synthesized at one time (**Figure 1.4**) and then be submitted for pharmacological assay in a variety of forms.[17]

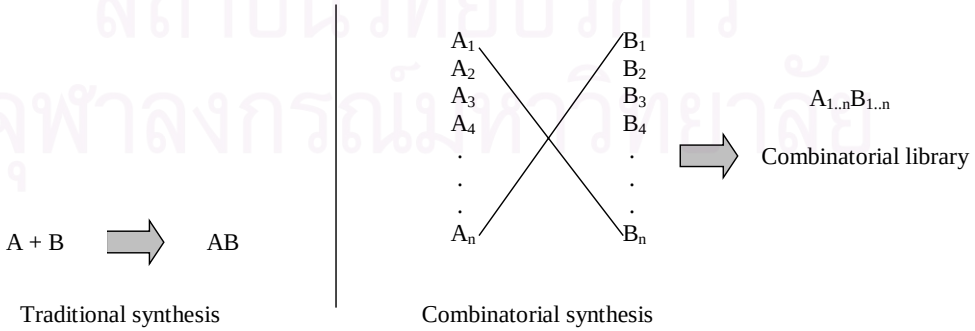


Figure 1.4: Comparing traditional and combinatorial synthesis, the former generally produces only one compound at a time, but combinatorial methods provide the potential to produce a range of products.

The key feature of combinatorial chemistry was that the synthesis was designed to generate a range of analogous compounds using similar reaction conditions, either in the same reaction vessel (mixture synthesis) or individually in parallel fashion (parallel synthesis).[17] Both of the mixture and parallel synthesis could be carried out in solution[18] or solid-phase.[19]

Technique of synthesis combinatorial library

1. Split synthesis method[20-23]

The basic strategy for synthesizing a combinatorial chemical library by the split synthesis technique is outlined in **Figure 1.5**. Principle of the split synthesis is to divide, couple and recombine. This technique was initially developed for peptide synthesis on solid phase but was later found very wide application including solution phase synthesis. The polystyrene beads which are solid support are divided into a number of aliquots of equal size and each aliquot is coupled to a different monomer (A, B or C). In the case of a combinatorial peptide library, the monomers would be protected amino acids. When coupling is complete on each aliquot of resin, the excess reagents are removed by washing with an appropriate solvent and the aliquot recombined and thoroughly mixed. This process of dividing the resin into aliquots, quantitatively coupling the monomer units separately on to each aliquot, and washing the resin before recombining and thoroughly mixing, may be repeated several times depending on the size of the combinatorial library required and the capacity of the resin to accommodate all members of the library. If the same set of monomers are used in each round of the coupling, a complete combinatorial library of oligomers will be obtained. When three monomers are used (A, B and C), two rounds of coupling gives a combinatorial library of $3^2 = 9$ members and three rounds gives a library of $3^3 = 27$ members. It is evidenced that this method can save a lot of time in synthesis.

Furthermore, since division of a single resin bead cannot be made, one resin bead will contain only one structure, the technique is also known as one-bead-one compound approach which facilitate the screening, e.g. by selection with labelled enzymes which will enable the most active compound to be identified while it is still attaching to the bead. Synthesis of combinatorial library in solution phase, however, gains no advantage in this respect since the library will be obtained as statistically distributed mixture of compounds therefore it must be used in conjunction with specialized screening techniques such as iterative deconvolution.

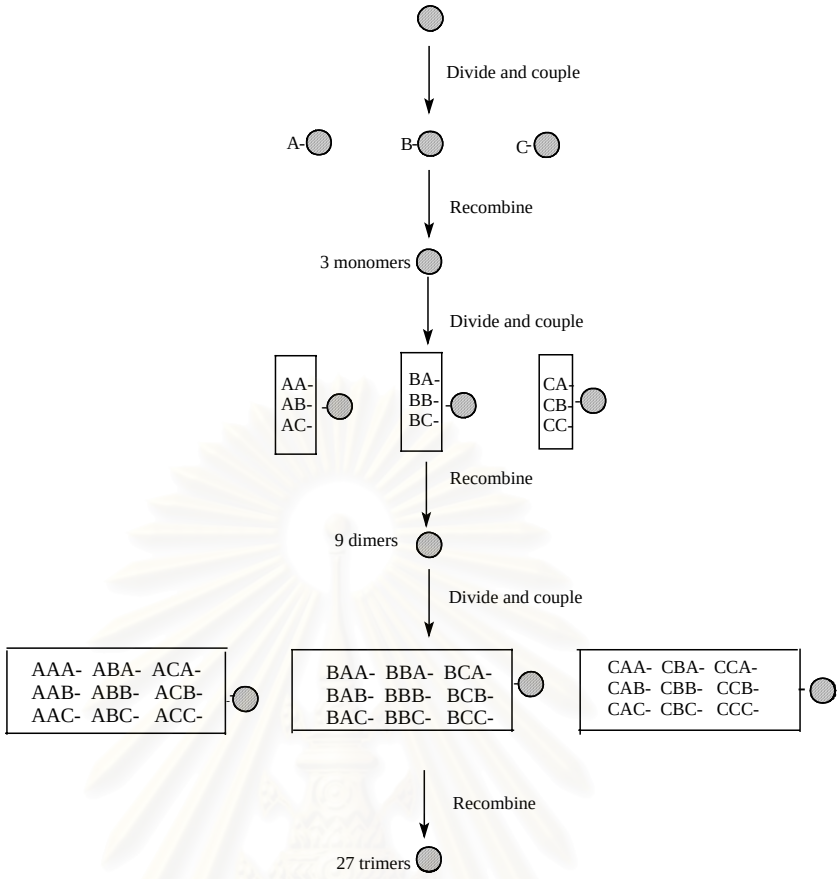


Figure 1.5: The split synthesis method of preparing a combinatorial library. In this illustration only three synthetic cycle with three monomers (A, B, and C) are used, giving a combinatorial library of 27 trimeric variants.

2. Parallel synthesis method

This is the other strategy to produce combinatorial library. Unlike the split synthesis, parallel synthesis will generate individual compounds. As a result, the number of synthesis would be the same as number of product. Consequently, this technique was more appropriate for semi-automated synthesis machine. However, the advantage of the technique is each member in the library can be prepared in larger amount and purer than those from mixture synthesis. Identifying the most active compound from this library is also more straightforward than from split synthesis.

Combinatorial chemistry was initially developed for peptide synthesis,[24-25] but its use was later extended to the synthesis of a range of small organic molecules.[26] More or less all structural type of compounds have been synthesized by combinatorial approach. Although compounds obtained from synthesis on solid-phase were easy to

screen and identify the active molecule, process of this synthesis method had difficulty in creating and cleaving the linkage between solid support and substrate. It also requires expensive solid support and sophisticated techniques. Therefore, solution phase synthesis would be preferred in this research. As a result, technique of screening to identify the most active compound from library (mixture) had to be used.

Screening combinatorial libraries

1. Enzyme selection technique

The screening of a combinatorial library for the highest affinity variant with a biological target was the commonest type of assay. By adding the specific labelled enzyme to the library beads (if it was solid phase synthesis). The enzyme would bind to the active compound on the bead and show difference between the active and inactive beads such as colour fluorescent properties associated with the labelling technique used. The bead that showed these properties would be separated and cleaved compound from bead to identify the structure of the active compound. The strategy can also be applied to solution phase screening, but are less convenient and therefore other methods such as iterative deconvolution are superior in this respect.

2. Iterative deconvolution[27]

The iterative deconvolution strategy has been extensively investigated in the last decade. It is one of the most powerful method in identifying the active molecules from combinatorial libraries. This method consists of the screening of compound pools (libraries), identifying the active pool(s), re-synthesizing and re-screening sublibraries (smaller pools). The structure of compound as known present in each pool, therefore leading to the identification of the active library member(s) without the need for separated identification step. Diagram of this method was illustrated in **Figure 1.6**

In this work, synthesis of the library will be came out in solution phase with the use of split synthesis technique and the most active compounds will be identified by iterative deconvolution technique since they require less advanced technique and sophisticated equipment while can still deliver reliable results.

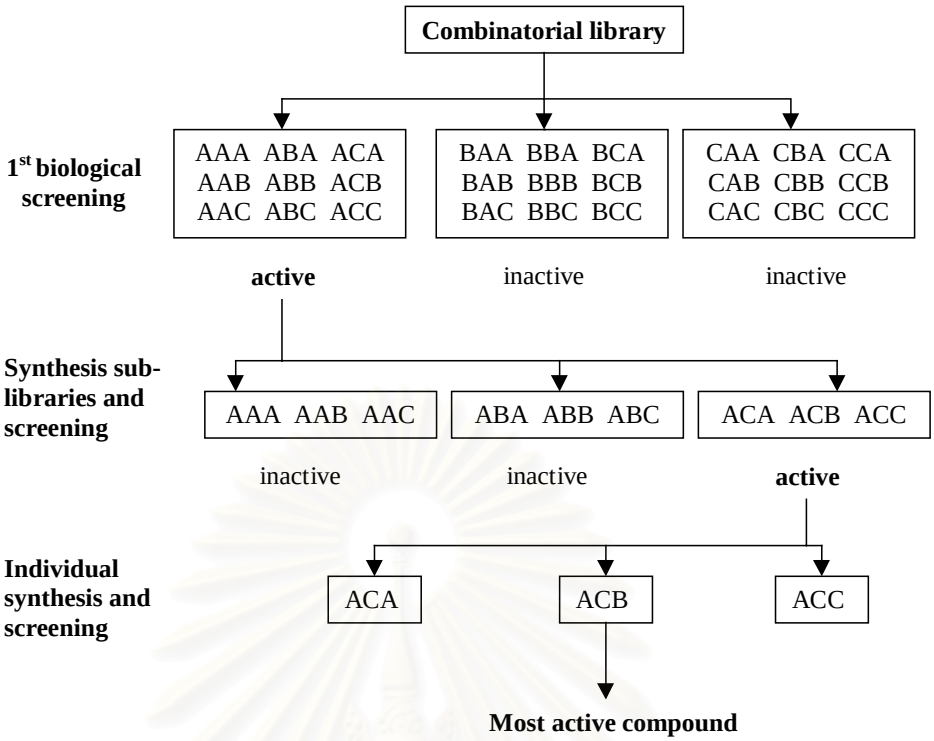
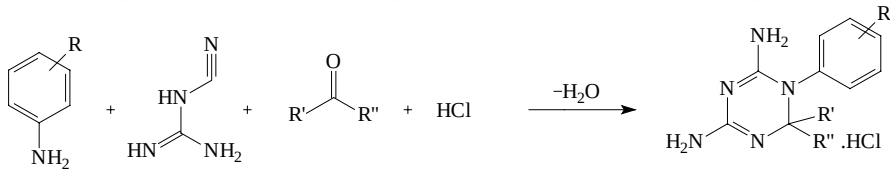


Figure 1.6: Step of iterative deconvolution

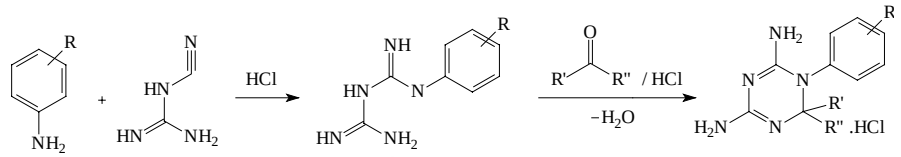
1.1.3 Chemical synthesis of dihydrotriazines

Even though combinatorial synthesis offers many advantages, this synthesis must limit itself to very efficient reactions in order to produce high quality libraries. In case of synthesis analogues of Cyc or derivatives of 4,6-diamino-1,2-dihydro-1,3,5-triazine, there were three methods available. In 1956, Modest reported three-component[28] and two-component[29] synthesis to synthesize derivatives of 4,6-diamino-1,2-dihydro-1,3,5-triazine. Three-component synthesis was the condensation reaction of the arylamine, dicyanodiamide and ketone or aldehyde under acidic condition (**Scheme 1.6**).



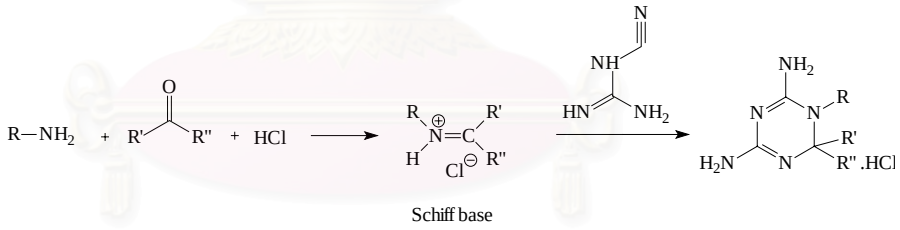
Scheme 1.6: Three-component synthesis

Two-component synthesis was the condensation of an arylbiguanide and a ketone or an aldehyde under acidic condition (**Scheme 1.7**). The arylbiguanide was generated from arylamine and dicyanodiamide in the presence of acid.



Scheme 1.7: Two-component synthesis

In 1964, Newman and Moon reported synthesis of 4,6-diamino-1,2-dihydro-1,3,5-triazine from the reaction of Schiff bases with dicyanodiamide.[30] The Schiff base, which was generated by reacting amine compound with ketone or aldehyde, and then reacted with dicyanodiamide (**Scheme 1.8**) in the presence of an acid catalyst. Not only arylamines but alkylamines can also be used as starting material in this method. While the three- and two-component synthesis were limited to arylamine as the amine component.



Scheme 1.8: The reaction of Schiff bases with dicyanodiamide

However, each synthesis method had its own limitation. The three-component synthesis proceeds well with aromatic aldehydes in place of ketones but fails with aliphatic aldehydes under the usual condition of the reaction. Both three- and two-component syntheses failed with sterically hindered ketone. The last method is not applicable to Schiff-base derived from enonizable aldehydes or ketones. As a result, the existing synthesis methods are not appropriate for solution phase combinatorial synthesis of derivatives of 4,6-diamino-1,2-dihydro-1,3,5-triazine. One of the goal in this work is, therefore, to find an appropriate condition for such synthesis.

1.2 Goal of research

The objectives of this research were to develop a method for the synthesis of of 4,6-diamino-1,2-dihydro-1,3,5-triazine libraries, and to identify and characterize the compounds those showed the best inhibition constants (K_i) against wild-type and A16VS108T mutant DHFRs of *P. falciparum* using combinatorial techniques.

CHAPTER II

EXPERIMENTAL

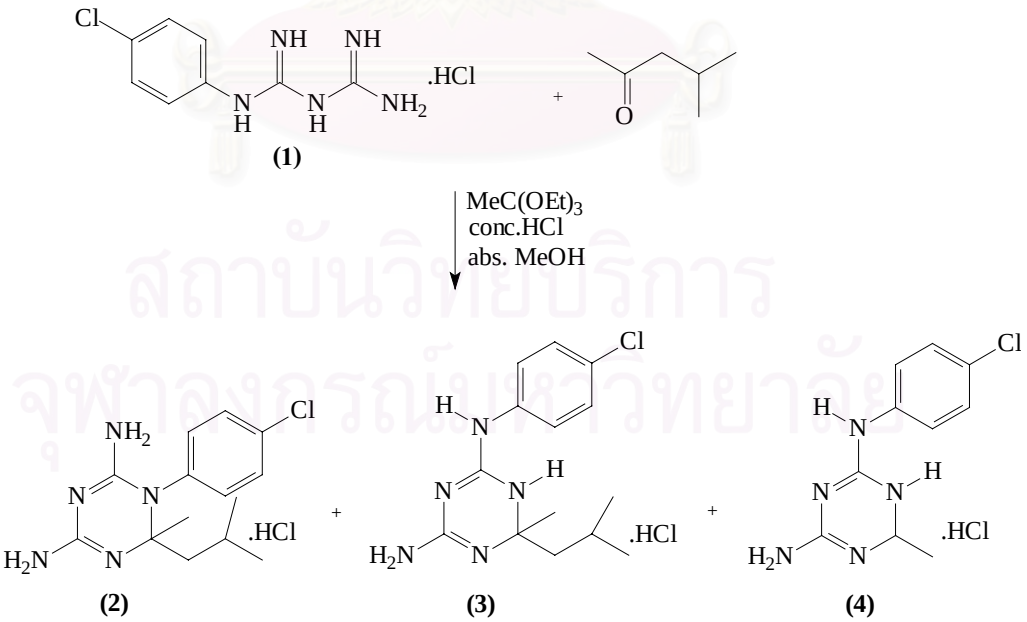
2.1 General

Thin layer chromatography was performed on Merck D.C. silica gel 60 F_{254} 0.2 mm precoated aluminium plates (cat no.1.05554). Removal of solvents was carried out on Buchi rotary evaporator attached to water aspirator. The weight of all substances was determined on a Metler AT200 electrical balance. ^{1}H NMR spectra were recorded in an appropriate deuterated solvent ($CDCl_3$, DMSO or D_2O) at 200 MHz (^{1}H) on a Bruker ACx200 NMR spectrometer. Chemical shift (δ) was reported in part per million (ppm, δ) and coupling constants (*J*) were in Hz. Elemental analyses were performed by Ms. Amporn Ungpakornkaew at Chulalongkorn Research Equipment Center on a Perkin-Elmer CHN analyzer model PE 2400 series II. The Metrohm 744 pH meter (Switzerland) was used for measuring the pH value of HPLC buffer. Reverse phase HPLC experiments were performed on Water™ 600 controller system equipped with a gradient pump Water™ 600 and Water™ 996 photodiode array detector; optionally alternate to Water™ 717 plus autosampler (20 μL sample size) (Millipore, USA). A Hypersil™ 5 μm BDS C_{18} HPLC column (4.6x250 mm) was used for analytical purpose. Peak monitoring and data processing were performed on Compaq Prolinea 486 compatible computer operating a Millennium Version 2.1 software. MALDI-TOF mass spectra of all sample was analyzed by Ms. Nathiga Panchan and Ms. Netnapa Chareonsethakul on Bruker BIFLEX™ using doubly recrystallized α-cyano-4-hydroxy cinnamic acid (CCA) as matrix, and calibrated with human angiotensin II (M+H,1047). Trifluoroacetic acid (0.1%) in acetonitrile:water (70:30) was used as diluting agent for preparation of all MALDI-TOF samples. ESI mass spectra of some combinatorial library samples were analysed by Mr. Sittichok Sittichotpong on Fisons Instrument VG TRIO 2000. Inhibition constants (K_i) of all sample against the wild-type and A16VS108T mutant DHFRs of *P.falciparum* were analyzed by Dr. Sumalee Kamchonwongpaisan and Ms. Duenpen Japroong at The National Science and Tech- nology Development Agency (NSTDA).

2.2 Chemicals

All chemicals were purchased from Fluka, Merck or Aldrich Chemical Co., Ltd., and were purified as appropriate depending on reaction condition and purposes. Absolute methanol, absolute ethanol, acetone, popan-2-ol and diethyl ether were analytical reagent grade, purchased from BDH. Acetonitrile for HPLC experiment was HPLC grade, obtained from BDH and was filtered through a membrane filter (13 mm ∅, 0.45 μm Nylon Lida) before used. Deionized water was obtained from Milli - pore system. Triethylammonium acetate buffer (0.1 M, pH 7) was used as mobile phase for HPLC experiments and prepared by dissolving 10.19 g of triethylamine in 950 mL deionized water and then equimolar glacial acetic acid was added and then adjusted to 7.0 (determined by pH meter). After that the volume of the solution was then adjusted to 1000 mL with deionized water and was filtered through a membrane filter. All samples to be analyzed by HPLC experiments were diluted by deionized water acetonitrile or mixture thereof.

2.3 Optimization of reaction condition for synthesis of 4,6-diamino-1,2-dihydro-1,3,5-triazine



A reaction between 4-chlorophenylbiguanide hydrochloride and methyl isobu-

tyl ketone (MIBK) was used as a model. The reaction was monitored by reverse phase HPLC (mobile phase = acetonitrile: 0.1 M triethylammonium acetate pH 7.0) with gradient elution (15%-60% MeCN). Three parameters were optimized, including ratio of reactants, amounts of acid catalyst and temperature.

HPLC gradient system

Flow rate = 1 mL/min

Solvent A = acetonitrile

Solvent B = 0.1 M Triethylammonium acetate

A:B (15:85) for 15 min then linear gradient to A:B (60:40) during 15 min then to 100% A in 5 min before revert back to A:B (15:85)

HPLC condition

UV spectrum of all chromatograms were collected from 200 to 400 nm but the chromatograms shown were recorded at 254 nm.

2.3.1 Optimization of reactants ratio

The ratio of reactants was optimized by varying the amounts of MIBK and triethyl orthoacetate; while the amount of 4-chlorophenylbiguanide hydrochloride was kept constant at 0.2481 g (1 mmol).

2.3.1a Varying amount of MIBK

In the first reaction, to 4-chlorophenylbiguanide hydrochloride 0.2481 g (1 mmol) in a 25 mL round-bottom flask was added 5 mL of absolute MeOH, 0.19 mL of MIBK (1.5 mmol), 0.61 mL triethyl orthoacetate (TEOA) and 0.045 mL of conc.HCl. The flask was stoppered and the mixture was stirred in an oil bath at 45 $^{\circ}$C. The volume of all reactants were kept constant in all subsequent experiments except for the volume of MIBK which was varied to 0.31 mL (2.5 mmol), 0.63 mL (5 mmol) and 1.24 mL (10 mmol) in the second, third and fourth experiment respectively. The relative proportions of each component as determined from HPLC chromatogram analysis were as shown in **Table 2.1-2.4**.

Table 2.1: % Peak areas of **(1)-(4)** according to HPLC analysis; when 0.19 mL (1.5 eq.) of MIBK was used

Time	**% Peak areas**[a]			
(hr)	**(1)**	**(2)**	**(3)**	**(4)**
0.5	91.8	7.7	0.5	0.0
1.0	86.0	12.8	1.2	0.0
2.0	77.8	19.7	2.5	0.0
3.0	69.2	28.1	2.1	0.5
4.0	65.2	31.7	2.5	0.6
7.0	55.9	39.5	3.7	0.9
10.0	50.9	42.7	4.8	1.5
13.0	49.1	43.0	6.0	1.9
18.0	50.5	38.8	8.1	2.6
23.0	49.3	38.9	8.8	3.0
28.0	48.1	38.1	10.3	3.5
33.0	47.1	37.4	11.6	3.9
38.0	46.0	36.9	12.8	4.3
43.0	45.3	36.0	14.0	4.7
48.0	44.4	35.3	15.2	5.1
51.5	45.8	34.0	15.2	5.0

[a] % Peak area = $\frac{\text{Peak Area x 100}}{\text{Sum of Peak Area}}$

the difference in ε of each compound was ignored

Table 2.2: % Peak areas of **(1)-(4)** from HPLC analysis; when 0.31 mL (2.5 eq.) of MIBK was used

Time	**% Peak areas**			
(hr)	**(1)**	**(2)**	**(3)**	**(4)**
1.0	87.8	11.2	1.0	0.0
3.0	73.6	24.3	1.6	0.5
6.0	57.9	37.2	3.9	1.0
10.0	49.4	42.7	6.5	1.5
12.0	46.2	45.3	6.9	1.6
29.0	41.0	43.8	12.3	2.9
48.5	37.8	38.4	19.6	4.2
52.5	36.8	38.1	20.6	4.6

Table 2.3: % Peak areas of **(1)-(4)** from HPLC analysis; when 0.63 mL (5 eq.) of MIBK was used

Time	**% Peak areas**			
(hr)	**(1)**	**(2)**	**(3)**	**(4)**
1.0	77.4	21.4	1.1	0.0
3.0	43.1	51.9	4.5	0.5
6.0	25.0	67.5	6.7	0.8
10.0	19.2	70.6	9.2	1.0
12.0	17.5	70.9	10.5	1.0
29.0	19.6	60.6	18.0	1.8
48.5	20.4	51.5	25.6	2.5
52.5	19.2	50.8	27.3	2.7

Table 2.4: % Peak areas of **(1)-(4)** from HPLC analysis; when 1.24 mL (10 eq.) of MIBK was used

Time (hr)	% Peak areas			
	(1)	**(2)**	**(3)**	**(4)**
1.0	77.2	20.5	2.3	0.0
3.0	37.1	57.2	5.4	0.4
6.0	18.3	72.6	8.4	0.7
10.0	14.1	73.8	11.5	0.7
12.0	14.0	73.1	12.0	0.9
29.0	13.1	65.1	20.6	1.1
42.5	15.0	54.0	29.4	1.6
52.5	14.0	51.7	32.5	1.7

2.3.1b Varying amount of TEOA

In the first reaction, to 4-chlorophenylbiguanide hydrochloride 0.2481 g (1 mmol) in a 25 mL round-bottom flask was added 5 mL of absolute MeOH, 0.63 mL of MIBK (5 mmol), 0 mL of TEOA and 0.045 mL of conc.HCl. The flask was stoppered and the mixture was stirred in oil bath at 45 $^{\circ}$C. The volume of all reactants were kept constant in all subsequent experiments except for the volume of TEOA which was varied to 0.35, 0.75 and 1 mL respectively. The results were as shown in **Table 2.5-2.8**.

Table 2.5: % Peak areas of **(1)-(4)** from HPLC analysis; when TEOA were not used in the reaction

Time	**% Peak areas**			
(hr)	**(1)**	**(2)**	**(3)**	**(4)**
1.0	99.2	0.8	0.0	0.0
4.0	97.1	2.9	0.0	0.0
10.0	92.0	6.8	1.1	0.0
22.5	83.5	13.7	2.8	0.0
30.0	78.9	16.8	4.3	0.0
48.0	74.7	24.5	0.8	0.0
63.0	64.5	26.2	9.3	0.0

Table 2.6: % Peak areas of **(1)-(4)** from HPLC analysis; when TEOA was 0.35 mL/mmol

Time	**% Peak areas**			
(hr)	**(1)**	**(2)**	**(3)**	**(4)**
1.0	94.8	5.2	0.0	0.0
4.0	95.1	1.8	2.1	1.0
10.0	66.6	28.3	3.5	1.6
12.0	64.1	30.1	4.0	1.8
24.5	50.0	40.0	7.1	2.8
29.5	46.6	40.5	9.5	3.4
52.5	37.8	42.4	14.9	4.8

Table 2.7: % Peak areas of **(1)-(4)** from HPLC analysis; when TEOA was 0.75 mL/mmol

Time	**% Peak areas**			
(hr)	**(1)**	**(2)**	**(3)**	**(4)**
1.0	53.6	42.6	3.8	0.0
4.0	16.2	75.7	7.5	0.6
10.0	16.0	73.3	10.2	0.5
12.0	15.3	73.0	11.0	0.7
24.5	15.5	67.6	15.7	1.1
29.5	19.5	61.7	17.8	1.0
52.5	13.1	59.5	25.7	1.7

Table 2.8: % Peak areas of **(1)-(4)** from HPLC analysis; when TEOA was 1.00 mL/mmol

Time	**% Peak areas**			
(hr)	**(1)**	**(2)**	**(3)**	**(4)**
1.0	28.7	65.2	5.6	0.4
4.0	11.2	78.9	8.9	1.0
10.0	10.6	77.4	11.6	0.4
12.0	11.2	75.3	12.4	1.0
24.5	7.3	74.6	17.0	1.0
29.5	6.9	71.8	20.3	1.0
52.5	5.5	64.5	28.8	1.3

2.3.2 Optimization of amounts of acid catalyst used

This parameter was optimized by varying amount of conc.HCl in the reaction condition. The reaction condition was 4-chlorophenylbiguanide hydrochloride 0.2481 g (1 mmol), 5 mL of absolute MeOH, 0.63 mL of MIBK (5 mmol), 0.75 mL of TEOA and reaction temperature as 45 °C (best condition from **2.3.1b**) but amount of conc.HCl was changed from 0.045 mL to 0.023mL. The result was shown in **Table 2.9**.

Table 2.9: % Peak areas of **(1)-(4)** from HPLC analysis; when HCl was 0.023 mL

Time	**% Peak areas**			
(hr)	**(1)**	**(2)**	**(3)**	**(4)**
1.0	75.2	23.5	0.7	0.6
4.0	16.1	72.1	11.1	0.7
10.0	2.4	82.0	14.6	1.0
25.0	2.0	75.6	21.4	1.1
32.0	2.4	73.4	23.0	1.2
52.0	2.2	67.3	29.5	1.0

2.3.3 Optimization of temperature

The reaction condition of 4-chlorophenylbiguanide hydrochloride 0.2481 g (1 mmol), 5 mL of absolute MeOH, 0.63 mL of MIBK (5 mmol) and 0.75 mL of TEOA (best condition from **2.3.1b**) was used but temperature that was changed to 30 °C (room temperature) and 64 °C (in oil bath) the first and second experiment respectively. The results were shown in **Table 2.10-2.11**.

Table 2.10: % Peak areas of **(1)-(4)** from HPLC analysis: at reaction temperature = 30°C

Time (hr)	% Peak areas			
	(1)	**(2)**	**(3)**	**(4)**
1.0	90.5	8.6	0.8	0.0
4.0	57.7	39.0	3.4	0.0
10.0	24.6	70.5	4.9	0.0
23.5	12.7	80.5	6.8	0.0
29.5	13.7	79.3	7.0	0.0
47.0	11.8	79.6	8.6	0.0

Table 2.11: % Peak areas of **(1)-(4)** from HPLC analysis: at reaction temperature = 64 °C

Time (hr)	% Peak areas			
	(1)	**(2)**	**(3)**	**(4)**
1.0	20	71	9	0
2.5	17	69	15	0
5.5	20	54	25	0
8.2	28	39	32	1

2.3.4 The optimized condition

From experiment **2.3.1a, 2.3.1b, 2.3.2** and **2.3.3** the best reaction condition for 1 mmol scale of 4-chlorophenylbiguanide hydrochloride was 5 mmol of MIBK, 0.75 mL of TEOA and 0.023 mL of conc.HCl in absolute methanol at 30 °C (room temperature) for 24 hrs. Percent peak area of each compounds after optimization were shown in **Table 2.12**.

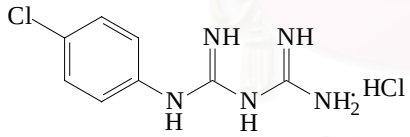
Table 2.12: % Peak area of **(1)-(4)** from HPLC analysis under optimized condition

Time (hr)	% Peak areas			
	(1)	**(2)**	**(3)**	**(4)**
1.0	91.6	7.4	0.6	0.4
4.0	65.3	31.9	2.3	0.4
10.0	13.6	79.4	6.5	0.5
24.0	4.5	88.0	7.3	0.3
52.0	4.6	83.4	11.9	0.2

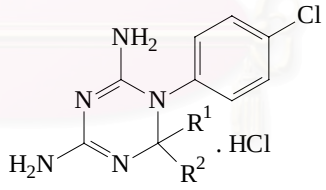
Remark: The data of % peak area in **Table 2.1-2.12** were obtained from the peak area of each compound from the HPLC chromatogram in **Table 1-12** respectively.

2.4 The effect of carbonyl compounds and substituent on benzene ring to the formation of 4,6-diamino-1,2-dihydro-1,3,5-triazine

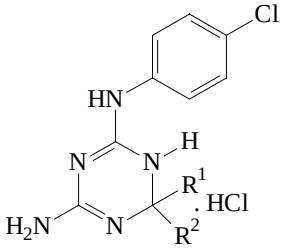
2.4.1 The effect of sterically hindered carbonyl compounds



(1)



(2)



(3)

	R¹	**R²**		**R¹**	**R²**
a)	$-CH_3$	$-^{i}Bu$	**d)**	$-^{n}Pr$	$-^{n}Pr$
b)	$-CH_3$	$-CH_3$	**e)**	-Et	$-^{n}Pen$
c)	$-CH_3$	$-^{i}Pr$	**f)**	-H	-Ph

The optimized condition **(2.3.4)** was used in the following experiments but MIBK was replaced by acetone (0.37 mL, 5 mmol), 3-methyl-2-butanone (0.54 mL, 5

mmol), 4-heptanone (0.7 mL, 5 mmol), 3-octanone (0.78 mL, 5 mmol) and benzaldehyde (0.51 mL, 5 mmol) respectively. The reactions were monitored by reverse phase HPLC (mobile phase and gradient system, see **2.3**). The results were shown in **Table 2.12** and **2.13-2.17**.

Table 2.13: % Peak areas of all substances from HPLC analysis when acetone was used as the carbonyl component.

Time	**% Peak areas**		
(hr)	**(1)**	**(2b)**	**(3b)**
1.0	95.6	4.2	0.2
4.0	80.5	18.4	1.1
10.0	43.3	54.5	2.2
24.3	4.4	91.5	4.2
33.3	1.8	93.8	4.4

Table 2.14: % Peak areas of all substances from HPLC analysis when 3-methyl-2-butanone was used as the carbonyl component.

Time	**% Peak areas**		
(hr)	**(1)**	**(2c)**	**(3c)**
1.0	98.8	1.2	0.0
4.0	94.0	5.4	0.7
10.0	84.9	13.7	1.4
24.0	63.8	33.2	3.0
52.0	37.9	56.3	5.8

Table 2.15: % Peak areas of all substances from HPLC analysis when 4-heptanone was used as the carbonyl component.

Time	% Peak areas		
(hr)	(1)	(2d)	(3d)
1.0	97.9	2.1	0.0
10.0	79.3	18.8	1.9
24.0	49.6	45.8	4.7
52.0	11.4	81.9	6.7
72.0	4.1	88.1	7.8

Table 2.16: % Peak areas of all substances from HPLC analysis when 3-octanone was used as the carbonyl component.

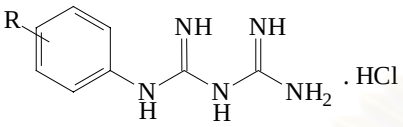
Time	% Peak areas		
(hr)	(1)	(2e)	(3e)
1.0	99.9	0.1	0.0
10.0	84.8	13.8	1.4
24.0	59.2	37.4	3.5
52.0	20.6	78.7	0.8
72.0	6.6	85.4	8.1

Table 2.17: % Peak areas of all substances from HPLC analysis when benzaldehyde was used as the carbonyl component.

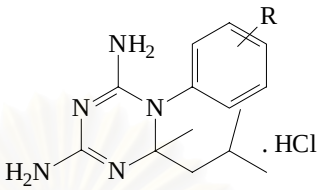
Time	% Peak areas		
(hr)	(1)	(2f)	(3f)
3.0	99.3	0.7	0.0
6.0	94.6	5.4	0.0
10.0	86.6	12.4	1.0
23.5	70.1	27.9	2.0
32.5	54.8	42.2	3.0
51.5	28.1	67.5	4.4
76.5	0.0	96.7	3.3

Remark: The data of % peak area in **Table 2.13-2.17** were obtained from the peak area of each compound from the HPLC chromatogram in **Table 13-17** respectively.

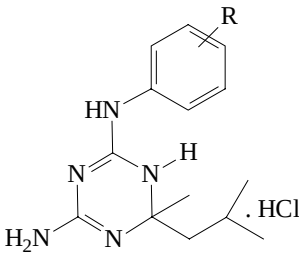
2.4.2 The effect of substitutent on benzene ring



(1)



(2)



(3)

	R		**R**
a)	4-Cl	**i)**	4-CH_3
h)	4-H	**j)**	3,4-Cl_2

The optimized condition **(2.3.4)** was used in the following experiments but 4-chlorophenylbiguanide hydrochloride **(1a)** was replaced by biguanide **(1h)**; 0.2138 g, **(1i)**; 0.2277 g and **(1j)**; 0.2826 g in following reactions respectively. The reactions were monitored by reverse phase HPLC (mobile phase and gradient system, see **2.3**). Results were as shown in **Table 2.12**, and **2.18-2.20**.

Table 2.18: % Peak areas of all substances from HPLC analysis when biguanide **(1h)** was used in the reaction

Time	**% Peak areas**		
(hr)	**(1h)**	**(2h)**	**(3h)**
1.0	89.5	10.0	0.5
4.0	68.0	29.8	2.3
10.0	43.3	56.7	0.0
24.0	28.6	66.7	4.6
52.0	16.0	79.6	4.4

Table 2.19: % Peak areas of all substances from HPLC analysis when biguanide **(1i)** was used in the reaction

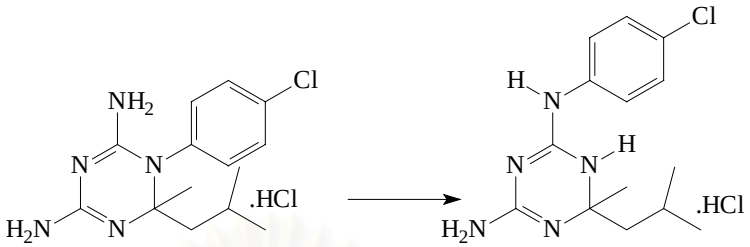
Time	**% Peak areas**		
(hr)	**(1i)**	**(2i)**	**(3i)**
1.0	95.6	4.1	0.2
4.0	80.5	17.4	2.1
10.0	43.7	51.7	4.6
24.0	7.2	86.1	6.7
52.0	2.1	90.0	7.9

Table 2.20: % Peak areas of all substances from HPLC analysis when biguanide **(1j)** was used in the reaction

Time	**% Peak areas**		
(hr)	**(1j)**	**(2j)**	**(3j)**
1.0	89.0	10.3	0.6
4.0	49.3	47.3	3.4
10.0	12.8	80.7	6.5
24.0	6.3	86.7	6.9
52.0	7.1	79.4	13.5

Remark: The data of % peak area in **Table 2.18-2.20** were obtained from the peak area of each compound from the HPLC chromatogram in **Table 18-20** respectively.

2.5 The effect of basicity and acidity of reaction medium in the rearrangement from 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazine (2) to 4-amino-6-anilino-1,2-dihydro-1,3,5-triazine (3)



4-Chlorophenyl-2-isobutyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2)** was used as a model compound. In the first experiment, 0.0034 g of **(2)** and 1.5 mL of triethylamine (7.2 mM in absolute MeOH) was added to a 5 mL round-bottom flask , and stirred at 45 °C. In the second experiment, 0.0052 g of **(2)** and 0.023 mL of conc.HCl in 1.5 mL of absolute MeOH was added to a round-bottom flask and stirred at 45 °C. In the control experiment, **(2)** 0.0033 g was stirred with 1.5 mL of absolute MeOH at 45 °C.

All reactions were monitored by reverse phase HPLC (mobile phase and gradient system, see **2.3**). Rearrangement from **(2)** to **(3)** was observed in all three cases. Especially, rearrangement of **(2)** to **(3)** was fastest in the presence of triethylamine (**Table 2.21**).

Table 2.21: % Peak areas of all substances from HPLC analysis of 1th-3rd experiment

Experiment	Time	% Peak areas		
	(hr)	(1)	(2)	(3)
Et_3N	0.0	0.0	100	0.0
	0.1	0.0	0.0	100
HCl	2.0	84.5	10.3	5.3
	20.5	82.8	2.6	14.5
	44.0	69.5	3.1	27.5
control	2.0	0.0	88.3	11.7
	20.5	0.0	79.3	20.7
	44.0	0.0	49.7	50.3

2.6 Synthesis of combinatorial libraries of 4,6-diamino-1,2-dihydro-1,3,5-triazine (used mixture of 11 ketone compounds)

Finding the optimal ratio of ketone mixture which produce equimolar mixture of dihydro- triazine compounds

Eleven carbonyl compounds including 2-pentanone, 2-hexanone, 2-heptanone, 5-methyl-2-hexanone, 2-octanone, 3-octanone, 3-pentanone, 4-methyl-2-pentanone, 2-butanone, acetone and 3-methyl-2-butanone were used in a model study. They were divided to three groups of mixture. The first group was mixture of 2-pentanone, 2-hexanone, 2-heptanone, 5-methyl-2-hexanone, 2-octanone and 3-octanone. The second group was mixture of 3-methyl-2-butanone, 2-heptanone and 3-octanone. And the last group was acetone, 2-butanone, 3-pentanone, 4-methyl-2-pentanone, 2-heptanone and 3-octanone.

Preparing ketone mixture

1. The first group

1-a). 2-pentanone 0.53 mL:2-hexanone 0.62 mL:2-heptanone 0.70 mL:5-methyl-2-hexanone 0.70 mL:2-octanone 0.78 mL:3-octanone 0.78 mL were mixed together to produce a mixture of 2-pentanone:2-hexanone:2-heptanone:5-methyl-2-hexanone:2-octanone:3-octanone in 1.0:1.0:1.0:1.0:1.0:1.0 mole ratio

1-b). 2-pentanone 0.53 mL:2-hexanone 0.62 mL:2-heptanone 0.70 mL:5-methyl-2-hexanone 0.7 mL:2-octanone 0.78 mL:3-octanone 2.81 mL were mixed together to produce a mixture of 2-pentanone:2-hexanone:2-heptanone:5-methyl-2-hexanone:2-octanone:3-octanone in 1.0:1.0:1.0:1.0:1.0: 3.6 mole ratio

2. The second group

2-a). 3-methyl-2-butanone 0.27 mL:2-heptanone 0.35 mL:3-octanone 1.4 mL were mixed together to produce a mixture of 3-methyl-2-butanone:2-heptanone:3-octanone in 1.0:1.0:3.6 mole ratio

2-b). 3-methyl-2-butanone 0.84 mL:2-heptanone 0.28 mL:3-octanone 1.12 mL were mixed together to produce a mixture of 3-methyl-2-butanone:2-heptanone:3-octanone in 3.9:1.0:3.6 mole ratio

2-c). 3-methyl-2-butanone 0.8 mL:2-heptanone 0.28 mL:3-octanone 1.12 mL were mixed together to produce a mixture of 3-methyl-2-butanone:2-heptanone:3-octanone in 3.8:1.0:3.6 mole ratio

3. The third group

3-a). acetone 0.19 mL:2-butanone 0.23 mL:3-pentanone 0.27 mL:4-methyl-2-pentanone 0.32 mL:2-heptanone 0.35 mL:3-octanone 1.4 mL were mixed together to produce a mixture of acetone:2-butanone:3-pentanone:4-methyl-2-pentanone:2-heptanone:3-octanone in 1.0:1.0:1.0:1.0:1.0:3.6 mole ratio

3-b). acetone 0.09 mL:2-butanone 0.16 mL:3-pentanone 0.57 mL:4-methyl-2-pentanone 2.61 mL:2-heptanone 0.35 mL:3-octanone 1.4 mL were mixed together to produce a mixture of acetone:2-butanone:3-pentanone:4-methyl-2-pentanone:2-heptanone:3-octanone in 1.2:1.8:5.4:20.9:2.5:9.0 mole ratio

3-c). acetone 0.12 mL:2-butanone 0.16 mL:3-pentanone 0.51 mL:4-methyl-2-pentanone 0.37 mL:2-heptanone 0.35 mL:3-octanone 1.4 mL were mixed together to produce a mixture of acetone:2-butanone:3-pentanone:4-methyl-2-pentanone:2-heptanone:3-octanone in 1.7:1.8:4.8:3.0:2.5:9.0 mole ratio

3-d). acetone 0.18 mL:2-butanone 0.22 mL:3-pentanone 0.72 mL:4-methyl-2-pentanone 0.54 mL:2-heptanone 0.35 mL:3-octanone 1.4 mL were mixed together to produce a mixture of acetone:2-butanone:3-pentanone:4-methyl-2-pentanone:2-heptanone:3-octanone in 2.4:2.5:6.8:4.3:2.5:9.0 mole ratio

3-e). acetone 0.21 mL:2-butanone 0.27 mL:3-pentanone 0.86 mL:4-methyl-2-pentanone 0.44 mL:2-heptanone 0.35 mL:3-octanon 1.4 mL were mixed together to produce a mixture of acetone:2-butanone:3-pentanone:4-methyl-2-pentanone:2-heptanone:3-octanone in 2.9:3.0:8.1:3.5:2.5:9.0 mole ratio

3-f). acetone 0.21 mL:2-butanone 0.27 mL:3-pentanone 0.86 mL:4-methyl-2-pentanone 0.54 mL:2-heptanone 0.35 mL:3-octanone 1.4 mL were mixed together to produce a mixture of acetone:2-butanone:3-pentanone:4-methyl-2-pentanone:2-heptanone:3-octanone in 2.9:3.0:8.1:4.3:2.5:9.0 mole ratio

3-g). acetone 0.21 mL:2-butanone 0.27 mL:3-pentanone 0.86 mL:4-methyl-2-pentanone 0.7 mL:2-heptanone 0.35 mL:3-octanone 1.4 mL were mixed together to produce a mixture of acetone:2-butanone:3-pentanone:4-methyl-2-pentanone:2-heptanone:3-octanone in 2.9:3.0:8.1:5.6:2.5:9.0 mole ratio

Remark: Each carbonyl compounds was added into a 10 mL volumetric flask and the final volume was adjusted to 10 mL with absolute MeOH.

Five mmol of each mixture was pipetted into a 25 mL round-bottom flask containing 4-chlorophenylbiguanide hydrochloride 0.2481 g, triethyl orthroacetate 0.75 mL and conc.HCl 0.023 mL. Absolute MeOH was added to each reaction until the total volume was 5 mL. Reactions were stirred at room temperature for 48 hrs and monitored by reverse phase HPLC (mobile phase and gradient system, see **2.3**). HPLC analysis of all reactions revealed that ratio the of ketone mixtures, which gave equimolar distribution of products in the combinatorial library of dihydrotriazine compounds were **1-a**, **2-c** and **3-g** respectively (showed in **Figure 1, 2** and **3**)

Synthesis of combinatorial libraries of 4,6-diamino-1,2-dihydro-1,3,5-triazine (used mixture of 11 ketone compounds)

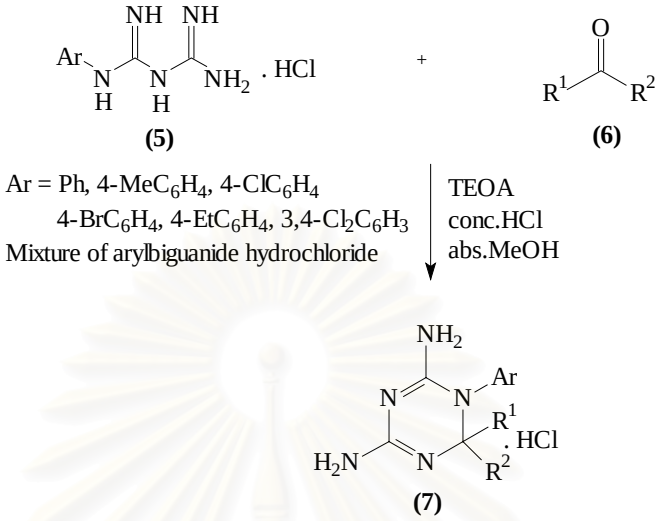
Preparing isokinetic mixture of 11 ketones

2-Pentanone (0.27 mL, 2.5 mmol), 2-hexanone (0.31 mL, 2.5 mmol), 5-methyl-2-hexanone (0.35 mL, 2.5 mmol), 3-octanone (1.4 mL, 9.0 mmol), 2-heptanone 0.35 mL (2.5 mmol), 2-octanone (0.39 mL, 2.5 mmol), of 3-pentanone (0.86 mL, 8.1mmol), 4-methyl-2-pentanone (0.70 mL, 5.6 mmol), 2-butanone (0.27 mL, 3.0 mmol), acetone (0.21 mL, 2.9 mmol) and of 3-methy-2-butanone (1 mL, 9.4mmol) were pipetted and diluted to 10 mL with absolute MeOH in 10 mL volumetric flask.

Typical procedure for synthesis of combinatorial libraries of 4,6-diamino-1,2-dihydro-1,3,5-triazine by two component method (for mixture of 11 ketone compounds)

To phenylbiguanide hydrochloride (0.2134 g, 0.9985 mmol) in a 25 mL round-bottom flask was added with ketone compounds mixture (1 mL, 5 mmol), absolute MeOH 4 mL, TEOA 0.75 mL and conc.HCl 0.0023 mL. The flask was stoppered and stirred at 30 °C (room temperature) for 2 days. The reaction was monitored by reverse phase HPLC (mobile phase and gradient system, see **2.3**). 4-methylphenylbiguanide hydrochloride (0.2279 g, 1 mmol), 4-chlorophenyl hydrochloride (0.2481 g, 1 mmol), 4-bromophenyl hydrochloride (0.2927 g, 1 mmol), 4-ethylphenyl hydrochloride (0.2417 g, 1 mmol) and 3,4-dichlorophenyl hydrochloride (0.2826 g, 1 mmol) were used in place of phenylbiguanide hydrochloride in other reactions.

2.7 Synthesis of combinatorial libraries of 4,6-diamino-1,2-dihydro-1,3,5-triazine (used mixture of 6 arylbiguanides)



Preparation of arylbiguanide hydrochloride mixture

Phenylbiguanide hydrochloride 0.2137 g, 4-methylphenylbiguanide hydrochloride 0.2276 g, 4-chlorophenylbiguanide hydrochloride 0.2481 g, 4-bromophenylbiguanide hydrochloride 0.2925 g, 4-ethylphenylbiguanide hydrochloride 0.2417 g and 3,4-dichlorophenylbiguanide hydrochloride 0.2826 g were mixed to give a biguanide mixture with a mole ratio of 1:1:1:1:1:1. The mixture was ground in a mortar to a fine homogeneous powder.

Typical procedure for synthesis of combinatorial libraries of 4,6-diamino-1,2-dihydro-1,3,5-triazine by two component method (for ketone and aromatic aldehyde as the carbonyl compound)

To the arylbiguanide hydrochloride mixture (average MW = 251.1, 0.2511 g, 1 mmol) in a 25 mL round-bottom flask was added 5 mL of absolute MeOH, the carbonyl compound (5 mmol), TEOA 0.75 mL and conc.HCl 0.023 mL. The flask was stoppered and the reaction was stirred at 30°C (room temperature) until a negative biguanide test was obtained (1-10 days). The crude product optained after solvent evaporation was washed with ether. The off-white solid precipitate was collected by filtration and washed again with Et_2O and air dried. The product was analyzed with reverse phase HPLC (HPLC gradient system as in **2.3**). Data of starting materials and % yield of products were shown in **Table 2.22**. The results from HPLC analysis of

sub-library **1-20** were as shown in **Figure 4-23** respectively. The data of mass spectral analysis of sub-libraries were as shown in **Table 2.23**.

Table 2.22: Synthesis data of combinatorial library

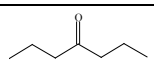
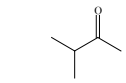
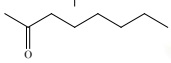
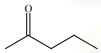
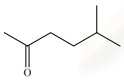
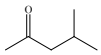
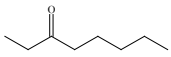
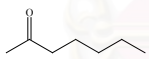
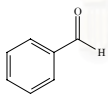
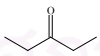
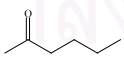
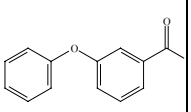
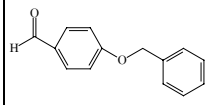
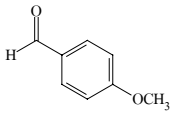
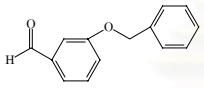
Sub-Library	(6)	time (hr)	weight(g)		mmol		$MW_{(average)}$ **(7)**.HCl	% yield
			(5)	**(7)**	**(5)**	**(7)**		
1		120	0.2512	0.2776	1.0006	0.8020	346.1454	80
2		125	0.2510	0.2314	0.9998	0.7274	318.1141	73
3		120	0.2510	0.2903	0.9998	0.8060	360.1611	81
4		120	0.2510	0.2328	0.9998	0.8012	290.5662	80
5		72	0.2509	0.2514	0.9994	0.7903	318.1141	79
6		72	0.2511	0.2855	1.0002	0.8248	346.1454	82
7		72	0.2515	0.2788	1.0018	0.8820	316.0985	88
8		24	0.2513	0.3136	1.0010	0.9442	332.1298	94
9		72	0.2509	0.3255	0.9994	0.9038	360.1611	90
10		72	0.2515	0.2872	1.0018	0.8297	346.1454	82
11		120	0.2511	0.3312	1.0002	0.9993	331.4344	100
12		113	0.2511	0.2992	1.0002	0.9405	318.1141	94
13		123	0.2512	0.3246	1.0006	0.9833	330.1141	98
14		113	0.2513	0.3123	1.0010	0.9403	332.1298	93
15		48	0.2512	0.2511	1.0006	0.8257	304.0985	82
16*		20	0.2510	0.2450	0.9998	0.8017	305.5985	80
17		>10 days	0.2525	0.3536	1.0057	0.8221	430.1091	81

Table 2.22: (continued)

Sub-library	**(6)**	time (hr)	weight(g)		mmol		$MW_{(average)}$ **(7)**.HCl	% yield
			(5)	**(7)**	**(5)**	**(7)**		
18		48	0.2508	0.3691	0.9990	0.8311	444.1247	83
19		72	0.2511	0.3247	1.0002	0.8821	368.0934	88
20		120	0.2527	0.2527	1.0065	0.5690	444.1247	57

* Isopropanol 5 mL was used instead absolute MeOH and noTEOA was used.

Table 2.23: $[MH]^+$ (MALDI-TOF) Data of Library ; ***** $[MH]^+$ (ESI)

$[MH]^+$**(7)**													**Figure**
Ar	**Ph**		**4-MeC$_6$H$_4$**		**4-ClC$_6$H$_4$**		**4-BrC$_6$H$_4$**		**4-EtC$_6$H$_4$**		**3,4-Cl$_2$C$_6$H$_3$**		
Sub-library	Obs.	Cal.	Obs.	Cal.	Obs.	Cal.	Obs.	Cal.	Obs.	Cal.	Obs.	Cal.	
1*	273.0	274.2	287.0	288.2	307.0	308.2	351.0, 353.0	352.1, 354.1	302.0	302.2	341.0	342.1	**24**
2*	246.0	246.2	260.0	260.2	279.2	280.1	325.0, 326.0	324.1, 326.1	274.0	274.2	314.0	314.1	**25**
3	288.3	288.2	302.3	302.2	322.3	322.2	366.0, 368.0	366.1, 368.1	316.3	316.3	356.3	356.1	**26**
4	218.0	218.1	232.0	232.2	251.9	252.1	296.0, 298.0	296.1, 298.1	246.0	246.2	287.0	286.0	**27**
5	246.0	246.2	260.4	260.2	280.0	280.1	324.0, 326.0	324.1, 326.1	274.4	274.2	314.0	314.1	**28**
6*	274.4	274.2	288.4	288.2	308.4	308.2	352.0, 354.0	352.1, 354.1	302.4	302.2	342.0	342.1	**29**
7	243.9	244.2	257.9	258.2	278.0	278.1	322.0, 324.0	322.1, 324.1	271.9	272.2	312.0	312.1	**30**
8	260.1	260.2	274.1	274.2	294.0	294.1	338.0, 340.0	338.1, 340.1	288.1	288.2	328.0	328.1	**31**
9	287.9	288.2	301.8	302.2	320.9	321.2	366.0, 368.0	366.1, 368.1	315.9	316.3	355.9	356.1	**32**
10	273.9	274.2	287.9	288.2	308.1	308.2	352.0, 354.0	352.1, 354.1	301.9	302.2	342.1	342.1	**33**
11	266.3	266.1	280.4	280.2	300.3	300.1	344.0, 346.0	344.1, 346.1	294.4	294.2	334.3	334.1	**34**
12*	245.0	246.2	259.0	260.2	280.0	280.1	324.0, 325.0	324.1, 326.1	274.0	274.2	314.0	314.1	**35**
13*	257.0	258.2	271.0	272.2	291.0	292.1	335.0, 337.0	336.1, 338.1	286.0	286.2	325.0	326.1	**36**
14*	259.0	260.2	273.0	274.2	293.0	294.1	338.0, 339.0	338.1, 340.1	287.0	288.2	327.0	328.1	**37**
15	232.2	231.2	246.2	246.2	266.2	266.1	310.0, 312.0	310.1, 312.1	260.3	260.2	301.1	300.1	**38**

Table 2.23: (Continued)

M.H$^+$**(7)**													**Figure**
Ar	**Ph**		**4-MeC$_6$H$_4$**		**4-ClC$_6$H$_4$**		**4-BrC$_6$H$_4$**		**4-EtC$_6$H$_4$**		**3,4-Cl$_2$C$_6$H$_3$**		
Sub-library	Obs.	Cal.	Obs.	Cal.	Obs.	Cal.	Obs.	Cal.	Obs.	Cal.	Obs.	Cal.	
16	204.0	204.1	218.0	218.1	237.9	238.1	281.9, 283.9	282.0, 284.0	232.0	232.2	271.9	272.0	**39**
17	356.0	358.2	370.0	372.2	399.0	392.1	436.0, 438.0	436.1, 438.1	384.0	386.2	323.9	426.1	**40**
18	371.7	372.2	358.6	386.2	405.5	406.9	449.9, 451.3	450.1, 452.1	399.6	400.2	349.3	440.1	**41**
19	296.0	296.2	310.0	310.2	330.0	330.1	373.8, 378.8	374.1, 376.1	324.0	324.2	363.9	364.1	**42**
20	372.2	372.2	386.2	386.2	406.1	406.9	450.0, 452.0	450.1, 452.1	400.2	400.2	440.0	440.1	**43**

2.8 Synthesis of individual 4,6-diamino-1,2-dihydro-1,3,5-triazine derivatives

Typical procedure for synthesis of 4,6-diamino-1,2-dihydro-1,3,5-triazine by two component method (for ketone as the carbonyl component).

To a suspension of the arylbiguanide.HCl (1 mmol) in absolute MeOH (5 mL) containing the ketone (5 mL) and triethyl orthoacetate (0.75 mL) was added the conc.HCl (0.023 mL). The reaction mixture was stirred at room temperature until a negative biguanide test was obtained (24 hr-120 hr) The crude product obtained after solvent evaporation was triturated with ether. The off-white solid precipitate was collected by filtration and washed again with Et_2O and air dried. Recrystallization from methanol ether by vapor diffusion technique afforded the pure products as white crystalline solids.

1-(4′-chlorophenyl)-2,2-dimethyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-1)**

Crude product 0.2823 g, 98 % (1.0 mmol scale); δ_H (DMSO, 200 MHz); 1.25 (2x3H, s, (CH_3)-2), 7.18 and 7.38 (2x2H, AB doublet, *J*=8.0 Hz, (aromatic C-H)-1), (**Figure 44**); *m/z* (MALDI-TOF) 252.4 $[MH]^+$

1-phenyl-2-methyl-2-propyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-2)**

Crude product 0.2612 g, yield 93 % (1.0 mmol scale); FOUND C, 55.46; H, 7.12; N, 24.75 % ($C_{13}H_{20}ClN_5$ requires C, 55.41; H, 7.15; N, 24.85 %); δ_H (D_2O, 200 MHz); 0.74 (3H, t, *J*=7.0 Hz, CH_2CH_2-2), 1.19 (3H, s, CH_3-2), 1.30-1.75 (2x2H, m, $CH(C\underline{H}_2)_2$-2), 7.20 and 7.60 (5H, 2xm, aromatic C-H), (**Figure 45**); *m/z* (MALDI-TOF) 246.2 $[MH]^+$

1-(4′-methylphenyl)-2-methyl-2-propyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-3)**

Crude product 0.2425 g, yield 82 % (1.0 mmol scale); FOUND C, 56.80; H, 7.51; N, 23.66 % ($C_{14}H_{22}ClN_5$ requires C, 56.84; H, 7.50; N, 23.67 %); δ_H (D_2O, 200 MHz); 0.72 (3H, t, *J*=7.0 Hz, CH_2CH_2-2), 1.15 (3H, s, CH_3-2), 1.22-1.65 (2x2H, m, 2xCH_2), 2.20 (3H, s, CH_3-4′), 7.05 and 7.20 (2x2H, AB doublet, *J*=8.0 Hz, aromatic C-H), (**Figure 46**); *m/z* (MALDI-TOF) 260.5 $[MH]^+$

1-phenyl-2,2-diethyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-4)**

Crude product 0.2042 g, yield 73 % (1.0 mmol scale); FOUND C, 55.41; H, 7.02; N, 24.85 % ($C_{13}H_{20}ClN_5$ requires C, 55.41; H, 7.15; N, 24.85 %); δ_H (D_2O, 200 MHz); 1.78 (6H, t, *J*=7.2 Hz, 2xCH_3), 1.50 (2x2H, m, 2xCH_2-2), 7.23 and 7.37 (5H, m, aromatic C-H), (**Figure 47**); *m/z* (MALDI-TOF) 246.2 $[MH]^+$

1-(3′,4′-dichlorophenyl)-2,2-diethyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-5)**

Crude product 0.3063 g, yield 88 % (1.0 mmol scale); FOUND C, 44.66; H, 4.99; N, 19.99 % ($C_{13}H_{28}Cl_3N_5$ requires C, 44.53; H, 5.17; N, 19.97 %);δ_H (D_2O, 200 MHz); 0.77 (6H, t, *J*=7.0 Hz, 2x$C\underline{H}_3CH_2$-2), 1.52 (2x2H, m, 2x$CH_3C\underline{H}_2$-2), 7.18 (1H, m, aromatic C-H), 7.48 (2H, m, aromatic C-H), (**Figure 48**); *m/z* (MALDI-TOF) 314.0 $[MH]^+$

1-phenyl-2,2-cyclopentylidene-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-6)**

Crude product 0.1701 g, yield 61 % (1.0 mmol scale); FOUND C, 55.92; H, 6.52; N, 25.16 % ($C_{13}H_{18}ClN_5$ requires C, 55.81; H, 6.48; N, 25.03 %); δ_H (D_2O, 200 MHz); 1.45 (2x2H, m, 2xCH_2-2), 1.72 (2x2H, m, 2xCH_2-2), 7.20 and 7.48 (5H, 2xm, aromatic C-H), (**Figure 49**); *m/z* (MALDI-TOF) 244.1$[MH]^+$

1-(4′-methylphenyl)-2,2-cyclopentylidene-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-7)**

Crude product 0.2297 g, yield 78 % (1.0 mmol scale); FOUND C, 57.29; H, 6.86; N, 23.90 % ($C_{14}H_{20}ClN_5$ requires C, 57.23; H, 6.86; N, 23.84 %); δ_H (D_2O, 200 MHz); 1.45 (2x2H, m, 2xCH_2-2), 1.75 (2x2H, m, 2xCH_2-2), 2.20 (3H, s, CH_3-4′), 7.08 and 7.20 (2x2HH, AB doublet, *J*=8.0 Hz, aromatic C-H), (**Figure 50**); *m/z* (MALDI-TOF) 258.1$[MH]^+$

1-(4′-ethylphenyl)-2-isopropyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-8)**

Crude product 0.1816 g, yield 59 % (1.0 mmol scale); FOUND C, 58.15; H, 7.78; N, 22.66 % ($C_{15}H_{24}ClN_5$ requires C, 58.15; H, 7.81; N, 22.60 %); δ_H (D_2O, 200

MHz); 0.70 and 0.90 (2x3H, 2xd, *J*=7.0 Hz, $(C\underline{H}_3)_2CH$-2), 1.02 (3H, t, *J*=7.2 Hz, CH_3-4′), 1.14 (3H, s, CH_3-2), 2.00 (1H, m, $(CH_3)_2C\underline{H}$-2), 2.50 (2H, q, *J*=15.2, 8.0 Hz, $C\underline{H}_2CH_3$), 7.02 (2H, m, aromatic C-H), 7.22 (2H, d, *J*=8.0 Hz, aromatic C-H), (**Figure 51**); *m/z* (MALDI-TOF) 275.5 $[MH]^+$

1-phenyl-2-isopropyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-9)**

Crude product 0.2353 g, yield 67 % (1.2 mmol scale); δ_H (D_2O, 200MHz); 0.72 and 0.90 (2x3H, 2xd, *J*=7.0 Hz, $(C\underline{H}_3)_2CH$-2), 1.14 (3H, s, CH_3-2), 2.02 (1H, m, $(CH_3)_2C\underline{H}$-2), 7.22 and 7.38 (5H, 2xm, aromatic C-H), (**Figure 52**); *m/z* (MALDI-TOF) 243.3 $[MH]^+$

1-(4′-chlorophenyl)-2-isopropyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-10)**

Crude product 0.1185 g, yield 38 % (1.0 mmol scale); FOUND C, 49.42; H, 5.93; N, 22.32 % ($C_{13}H_{19}Cl_2N_2$ requires C, 49.38; H, 6.06; N, 22.15 %); δ_H (D_2O, 200 MHz); 0.70 and 0.85 (2x3H, 2xd, *J*=7.0 Hz, $(C\underline{H}_3)_2CH$-2), 1.13 (3H, s, CH_3-2), 2.00 (1H, m, $(CH_3)_2C\underline{H}$-2), 7.25 and 7.85 (2x2H, AB doublet, *J*=8.0 Hz, aromatic C-H), (**Figure 53**); *m/z* (MALDI-TOF) 281.4 $[MH]^+$

1-(3′,4′-dichlorophenyl)-2-isopropyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-11)**

Crude product 0.2946 g, yield 84 % (1.0 mmol scale); δ_H (D_2O, 200 MHz); 0.70 and 0.85 (6H, 2xd, *J*=7.0 Hz, $2xCH_3$), 1.13 (3H, t, *J*=7.2 Hz, CH_3-2), 2.00 (1H, m, $C\underline{H}CH_3$), 7.15 (1H, m, aromatic C-H), 7.50 (2H, m, aromatic C-H), (**Figure 54**); *m/z* (MALDI-TOF) 315.6 $[MH]^+$

1-(4′-methylphenyl)-2-isobutyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride. H_2O **(2-12)**

Crude product 0.2314 g, yield 75 % (1.0 mmol scale); FOUND C, 55.18; H, 7.81; N, 21.42 % ($C_{15}H_{25}ClN_5+H_2O$ requires C, 54.95; H, 7.99; N, 21.36 %); δ_H (D_2O, 200 MHz); 0.73 (2x3H, d, *J*=7.2 Hz, $(C\underline{H}_3)_2CHCH_2$-2), 1.13 (3H, s, CH_3-2), 1.45 and 1.86 (2H, 2xdd, *J*=14.4, 6.0 Hz, $(CH_3)_2CHC\underline{H}_2$-2), 1.60 (1H, m, $(CH_3)_2$

C<u>H</u>CH_2-2), 2.22 (3H, s, CH_3-4′), 7.05 (2H, m, aromatic C-H), 7.15 (2H, d, *J*=8.0 Hz, aromatic C-H), (**Figure 55**); *m/z* (MALDI-TOF) 275.8 $[MH]^+$

1-(4′-chlorophenyl)-2-isobutyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride.H_2O **(2-13)**

Crude product 0.2049 g, yield 62 % (1.0 mmol scale); FOUND C, 48.42; H, 6.38; N, 20.04 % ($C_{14}H_{21}Cl_2N_5$+H_2O requires C, 48.28; H, 6.66; N, 20.11 %); δ_H (D_2O, 200 MHz); 0.73 (2x3H, d, *J*=7.2 Hz, (C<u>H</u>$_3$)$_2$CHCH_2-2), 1.13 (3H, s, CH_3-2), 1.45 and 1.83 (2xH, 2xdd, *J*=14.4, 6.4 Hz, $(CH_3)_2$CHC<u>H</u>$_2$-2), 1.55 (1H, m, $(CH_3)_2$ C<u>H</u>CH_2-2), 7.18 (2H, m, aromatic C-H), 7.38 (2H, d, *J*=8.0 Hz, aromatic C-H), (**Figure 56**); *m/z* (MALDI-TOF) 295.6 $[MH]^+$

1-(3′,4′-dichlorophenyl)-2-isobutyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-14)**

Crude product 0.2353 g, 53 % (1.2 mmol scale); δ_H (D_2O, 200 MHz); 0.91 (2x3H, d, *J*=7.2 Hz, (C<u>H</u>$_3$)$_2$CHCH_2-2), 1.26 (3H, s, CH_3-2), 1.52 (1H, m, $(CH_3)_2$C<u>H</u> CH_2-2), 1.76 (2H, d, *J*=7.5 Hz, $(CH_3)_2$CHC<u>H</u>$_2$-2), 7.39 and 7.80 (3H, 2x m, aromatic C-H), (**Figure 57**); *m/z* (MALDI-TOF) 328.0 $[MH]^+$

1-(3′,4′-dichlorophenyl)-2-isopentyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-15)**

Crude product 0.2803 g, yield 75 % (1.0 mmol scale); FOUND C, 47.51; H, 5.85; N, 18.45 % ($C_{15}H_{22}Cl_3N_5$ requires C, 47.57; H, 5.85; N, 18.49 %); δ_H (D_2O, 200 MHz); 1.08 (6H, d, *J*=7.2 Hz, (C<u>H</u>$_3$)$_2$CHCH_2CH_2-2), 1.18 (1H, m, $(CH_3)_2$C<u>H</u>$(CH_2)_2$-2), 1.20 (3H, s, CH_3-2), 1.65 (2H, m, $(CH_3)_2$CHC<u>H</u>$_2$CH_2-2), 1.75 (2H, m, $(CH_3)_2$CH CH_2C<u>H</u>$_2$-2), 7.24 (1H, m, aromatic C-H), 7.55 (2H, m, aromatic C-H), (**Figure 58**); *m/z* (MALDI-TOF) 341.9 $[MH]^+$

1-(4′-chlorophenyl)-2,2-dipropyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-16)**

Crude product 0.2349 g, yield 68 % (1.0 mmol scale); FOUND C, 52.24; H, 6.60; N, 20.40 % ($C_{15}H_{24}Cl_2N_5$ requires C, 52.33; H, 6.73; N, 20.34 %); δ_H (D_2O, 200 MHz); 0.65 (6H, t, *J*=7.0 Hz, 2xC<u>H</u>$_3$CH_2CH_2-2), 1.25 (4H, m, 2xCH_3C<u>H</u>$_2$CH_2-2),

1.45 (4H, m, 2x$CH_3CH_2C\underline{H}_2$-2),7.15 and 7.25 (2x2H, AB doublet, *J*=8.0 Hz, aromatic C-H), (**Figure 59**); *m/z* (MALDI-TOF) 308.2 $[MH]^+$

1-(3′,4′-dichlorophenyl)-2-methyl-2-pentyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-17)**

Crude product 0.3438 g, 91 % (1.0 mmol scale); δ_H (D_2O, 200 MHz); 0.66 (3H, t, *J*=7.0 Hz, $C\underline{H}_3(CH_2)_4$-2), 1.25 (3H, s, CH_3-2), 1.08, 1.52 and 1.74 (9H, 3xm, -$(CH_2)_4$-2), 7.17 (1H, m, (aromatic C-H)-1), 7.52 (2H, m, (aromatic C-H)-1), (**Figure 60**); *m/z* (MALDI-TOF) 341.8 $[MH]^+$

1-phenyl-2-ethyl-2-pentyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-18)**

Crude product 0.2557 g, yield 79 % (1.0 mmol scale); δ_H (D_2O, 200 MHz); 0.67 (3H, t, *J*=6.8 Hz, $C\underline{H}_3(CH_2)_4$-2), 0.72 (3H, t, *J*=7.0 Hz, $C\underline{H}_3CH_2$-2), 1.05-1.62 (8H, 4xm, 4xCH_2-2), 7.25 and 7.38 (5H, m, aromatic C-H), (**Figure 61**); *m/z* (MALDI-TOF) 288.5 $[MH]^+$

1-(4′-chlorophenyl)-2-ethyl-2-pentyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-19)**

Crude product 0.2274 g, yield 64 % (1.0 mmol scale); δ_H (D_2O, 200 MHz); 0.65 (3H, t, *J*=6.8 Hz, $C\underline{H}_3(CH_2)_4$-2), 0.80 (3H, t, *J*=7.0 Hz, $C\underline{H}_3CH_2$-2), 1.04-1.60 (5x2H, m, 5xCH_2-2), 7.15 and 7.45 (2x2H, AB doublet, *J*=8.0 Hz, aromatic C-H), (**Figure 62**); *m/z* (MALDI-TOF) 324.1 $[MH]^+$

1-phenyl-2-hexyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine. 0.5H_2O **(2-20)**

Crude product 0.2877 g, yield 89 % (1.0 mmol scale); FOUND C, 57.40; H, 8.39; N, 21.64 % ($C_{16}H_{26}ClN_5$+ 0.5H_2O. requires C, 57.73; H, 8.81; N, 21.04 %); δ_H (D_2O, 200MHz); 0.63 (3H, t, *J*=7.2 Hz, $C\underline{H}_3(CH_2)_5$-2), 1.05-1.73 (5x2H, 5xm, 5xCH_2-2), 1.15 (3H, s, CH_3-2), 7.18 and 7.38 (5H, 2xm, aromatic C-H) (**Figure 63**); *m/z* (MALDI-TOF) 288.5 $[MH]^+$

1-(4′-methylphenyl)-2-hexyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-21)**

Crude product 0.3044 g, yield 90 % (1.0 mmol scale); FOUND C, 60.18; H, 8.69; N, 20.86 % ($C_{17}H_{24}ClN_5$ requires C, 60.43; H, 8.35; N, 20.73 %); δ_H (D_2O, 200 MHz); 0.64 (3H, t, *J*=7.2 Hz, $C\underline{H}_3(CH_2)_5$-2), 1.05-1.70 (5x2H, 5xm, 5xCH_2-2), 1.17 (3H, s, CH_3-2), 2.20 (3H, s, CH_3-4′) 7.05 (2H, m, aromatic C-H), 7.20 (2H, d, *J*=8.0 Hz, aromatic C-H), (**Figure 64**); *m/z* (MALDI-TOF) 303.7 $[MH]^+$

1-(4′-ethylphenyl)-2-hexyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-22)**

Crude product 0.2945 g, yield 84 % (1.0 mmol scale); FOUND C, 61.44; H, 8.59; N, 19.89 % ($C_{18}H_{30}ClN_5$ requires C, 61.43; H, 8.59; N,19.90 %); δ_H (D_2O, 200MHz); 0.67 (3H, t, *J*=7.2 Hz, $C\underline{H}_3(CH_2)_5$-2), 1.05 (3H, t *J*=7.00 Hz, $CH_2C\underline{H}_3$-4′), 1.10-1.70 (5x2H, 5xm, 5xCH_2-2), 1.20 (3H, s, CH_3-2), 2.02 (2H, q, *J*=7.0 Hz, CH_2-4′), 7.12 (2H, m, aromatic C-H) 7.25 (2H, d, *J*=8.0 Hz, aromatic C-H), (**Figure 65**); *m/z* (MALDI-TOF) 361.5 $[MH]^+$

1-(4′-chlorophenyl)-2-hexyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-23)**

Crude product 0.3046 g, yield 85 % (1.0 mmol scale); FOUND C, 53.58; H, 6.99; N, 19.83 % ($C_{16}H_{25}Cl_2N_5$ requires C, 53.58; H, 7.03; N, 19.55 %); δ_H (D_2O, 200MHz); 0.65 (3H, t, *J*=7.2 Hz, $C\underline{H}_3(CH_2)_5$-2), 1.08-1.70 (5x2H, 5xm, 5xCH_2-2), 1.18 (3H, s, CH_3-2), 7.20 (2H, m, aromatic C-H), 7.40 (2H, d, *J*=8.0 Hz, aromatic C-H), (**Figure 66**); *m/z* (MALDI-TOF) 322.5 $[MH]^+$

1-(4′-bromophenyl)-2-hexyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-24)**

Crude product 0.3516 g, yield 86 % (1.0 mmol scale); δ_H (D_2O, 200 MHz); 0.67 (3H, t, *J*=7.2 Hz, $C\underline{H}_3(CH_2)_5$-2), 1.07-1.72 (5x2H, 5xm, 5xCH_2-2), 1.17 (3H, s, CH_3-2), 7.15 (2H, m, aromatic C-H), 7.55 (2H, d, *J*=8.0 Hz, aromatic C-H), (**Figure 67**); *m/z* (MALDI-TOF) 366.1 and 368.1 $[MH]^+$

1-(3′,4′-dichlorophenyl)-2-hexyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-25)**

Crude product 0.3618 g, yield 92 % (1.0 mmol scale); FOUND C, 48.89; H, 6.14; N, 17.87 % ($C_{16}H_{24}Cl_3N_5$ requires C, 48.93; H, 6.16; N, 17.83 %) δ_H (D_2O, 200MHz); 0.63 (3H, t, *J*=7.2 Hz, $C\underline{H}_3(CH_2)_5$-2), 1.02-1.70 (5x2H, 5xm, 5xCH_2-2), 1.68 (3H, s, CH_3-2), 7.13 and 7.47 (2x2H, 2xm, aromatic C-H), (**Figure 68**); *m/z* (MALDI-TOF) 356.4 $[MH]^+$

1-(4′-chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-26)**

Crude product 0.2318 g, 69 % (1.0 mmol scale); δ_H (DMSO, 200 MHz); 6.06 (1H, s, H-2), 7.16 and 7.43 (2x2H, AB doublet, *J*=8.0 Hz, (aromatic C-H)-1), 7.36 (5H, m, (aromatic C-H)-2), (**Figure 69**) ; *m/z* (MALDI-TOF) 300.4 $[MH]^+$

1-(4′-ethylphenyl)-2-(4′′-benzyloxy)phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-27)**

Crude product 0.3335 g, 93 % (0.8 mmol scale); FOUND C, 66.09; H, 6.23; N, 6.00 % ($C_{24}H_{26}ClN_5O$ requires C, 66.12; H, 6.01; N, 16.06 %); δ_H (DMSO, 200MHz); 1.03(3H, t, *J*=7.2 Hz, $CH_2C\underline{H}_3$-4′), 2.03(2H, q, *J*=7.0 Hz, CH_2-4′), 5.08 (2H, s, $C\underline{H}_2$-benzyl), 5.92 (1H, s, H-2), 7.00 and 7.40 (9H, 2xm, aromatic C-H), 7.24 (4H, d, *J*=8.0 Hz, aromatic C-H), (**Figure 70**); *m/z* (MALDI-TOF) 400.1 $[MH]^+$

1-(4′-bromophenyl)-2-(4′′-benzyloxy)phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-28)**

Crude product 0.4393 g, yield 75 % (1.2 mmol scale); FOUND C, 54.16; H, 4.53; N, 14.20 % ($C_{22}H_{24}ClN_5O$ requires C, 54.28; H, 4.35; N, 14.39 %); δ_H (DMSO, 200MHz); 5.08 (2H, s, $C\underline{H}_2$-benzyl), 6.00 (1H, s, H-2), 7.00, 7.08, 7.23 and 7.57 (4x2H, 4xd, *J*=8.0 Hz aromatic C-H), 7.38 (5H, m, aromatic C-H), 9.0 (1H, s, NH_2), (**Figure 71**); *m/z* (MALDI-TOF) 449.9 and 452.0 $[MH]^+$

1-phenyl-2-(4′′-benzyloxy)phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-29)**

Crude product 0.2254 g, 55 % (1.0 mmol scale); FOUND C, 64.54; H, 5.53; N,17.14 % ($C_{22}H_{22}ClN_5O$ requires C, 64.78; H, 5.44; N, 17.17 %); δ_H (DMSO, 200 MHz); 5.07 (2H, s, C<u>H</u>$_2$-benzyl), 5.97 (1H, s, H-2), 7.04 and 7.24 (2x2H, AB doublet, *J*=8.0 Hz, aromatic C-H), 7.14 and 7.40 (10H, 2xm, aromatic C-H), (**Figure 72**); m/z (MALDI-TOF) 372.6 $[MH]^+$

1-(4′-methylphenyl)-2-(4′′-benzyloxy)phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-30)**

Crude product 0.3098 g, 73 % (1.0 mmol scale); FOUND C, 65.50; H, 5.75; N,16.64 % ($C_{23}H_{24}ClN_5O$ requires C, 65.47; H, 5.73; N, 16.60 %); δ_H (DMSO, 200 MHz); 2.03 (3H, s, CH_3-4′), 5.08 (2H, s, C<u>H</u>$_2$-benzyl), 5.92 (1H, s, H-2), 7.01 (4H, d, *J*=8.0 Hz, aromatic C-H), 7.20 (4H, m, aromatic C-H), 7.34 (5H, m, aromatic C-H), (**Figure 73**); *m/z* (MALDI-TOF) 385.7 $[MH]^+$

1-(4′-chlorophenyl-2-(4′′-benzyloxy)phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-31)**

Crude product 0.3318 g, 75 % (1.0 mmol scale); FOUND C, 59.75; H, 4.35; N, 15.81 % ($C_{22}H_{21}Cl_2N_5O$ requires C, 59.74; H, 4.79; N, 15.83 %); δ_H (DMSO, 200 MHz); 5.07 (2H, s, C<u>H</u>$_2$-benzyl), 5.99 (1H, s, H-2), 6.99 and 7.23 (2x2H, AB doublet, *J*=8.0 Hz, aromatic C-H), 7.24 and 7.46 (2x2H, AB doublet, *J*=8.0 Hz, aromatic C-H), 7.41 (5H, m, aromatic C-H), (**Figure 74**); *m/z (*MALDI-TOF) 405.7 $[MH]^+$

1-(3′,4′-dichlorophenyl-2-(4′′-benzyloxy)phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-32)**

Crude product 0.3053 g, 64 % (1.0 mmol scale); FOUND C, 55.44; H, 4.18; N, 14.65 % ($C_{22}H_{20}Cl_3N_5O$ requires C, 55.42; H, 4.23; N, 14.69 %); δ_H (DMSO, 200 MHz); 5.08 (2H, s, C<u>H</u>$_2$-benzyl), 6.04 (1H, s, H-2), 6.99 and 7.26 (2x2H, AB doublet, *J*=8.0 Hz, aromatic C-H), 7.13 (5H, m, aromatic C-H), 7.59 (5H, m, aromatic C-H), (**Figure 75**); *m/z* (MALDI-TOF) 439.8 $[MH]^+$

Typical procedure for synthesis of 4,6-diamino-1,2-dihydro-1,3,5-triazine by two component method (for aldehyde as the component).

To a suspension of the arylbiguanide.HCl (0.5-1 mmol) in an appropriate volume of absolute EtOH (3 mL) containing conc.HCl (0.023-0.045 mL) was added the aldehyde (2 eq.). The reaction mixture was heated at reflux until a negative biguanide test was obtained (30 min to several hours). On cooling in the fridge, a white solid precipitated which was collected by filtration and washed with EtOH (colde), acetone then Et_2O and dried air. Recrystallization from methanol by ether vapor diffusion afforded the pure product as white crystalline solids.

1-phenyl-2-(4′-(2″,4″,5″-trichlorophenoxypropyloxy)phenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-33)**

0.0560 g, yield 20 % (0.5 mmol scale); δ_H (DMSO, 200 MHz); 2.17 (2H, quintet, -$CH_2C\underline{H}_2CH_2$-), 4.10 (2H, t, *J*=7.5 Hz, -$OC\underline{H}_2$-), 4.25 (2H, t, *J*=7.5 Hz, -$OC\underline{H}_2$-), 5.96 (1H, d, H-2), 6.93 and 7.14 (2x2H, AB doublet, *J*=8.0 Hz, (aromatic C-H)-2), 7.10 (2H, d, *J*=8.0 Hz aromatic C-H), 7.21 (3H, m, aromatic CH), 7.50 and 7.80 (2xH, 2xs, (aromatic C-H)-2″,6″), (**Figure 76**); *m/z* (MALDI-TOF) 517.8 $[MH]^+$

1-(3′-chlorophenyl)-2-(4′-(2″,4″,5″-trichlorophenoxypropyloxy)phenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-34)**

0.196 g, yield 33 % (1.0 mmol scale); FOUND C, 48.89; H, 3.49; N, 11.85 % ($C_{24}H_{22}Cl_5N_5O_2$ requires C, 48.88; H, 3.76; N, 11.88 %); δ_H (DMSO, 200 MHz); 2.17 (2H, m, -$CH_2C\underline{H}_2CH_2$-), 4.08 (2H, t, *J*=7.5 Hz, -$OC\underline{H}_2$-), 4.25 (2H, t, *J*=7.0 Hz, -$C\underline{H}_2$O-), 6.01 (1H, s, H-2), 6.94 and 7.38 (2x2H, AB doublet, *J*=8.0 Hz, (aromatic C-H)-2), 7.00 (H, m, aromatic CH), 7.22 (1H, s, aromatic C-H), 7.27 (2H, d, *J*=8 Hz, aromatic C-H), 7.49 and 7.79 (2xH, 2xs, (aromatic C-H)-2″,6″), (**Figure 77**); *m/z* (MALDI-TOF) 554.3 $[MH]^+$

1-(4′-chlorophenyl)-2-(4′-(2″,4″,5″-trichlorophenoxypropyloxy)phenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-35)**

0.1815 g, yield 62 % (0.5 mmol scale); FOUND C, 48.87; H, 3.72; N, 11.88 % ($C_{24}H_{22}Cl_5N_5O_2$ requires C, 48.88; H, 3.76; N, 11.88 %); δ_H (DMSO, 200 MHz); 2.16 (2H, m, -$CH_2C\underline{H}_2CH_2$-), 4.10 (2H, t, *J*=7.0 Hz, -$OC\underline{H}_2$-), 4.25 (2H, t, *J*=7.0 Hz, -$C\underline{H}_2O$-), 5.96 (1H, s, H-2), 6.93 and 7.22 (2x2H, AB doublet, *J*=8.0 Hz, (aromatic C-H), 7.14 and 7.42 (2x2H, AB doublet, *J*=8.0 Hz, aromatic C-H), 7.49 and 7.79 (2xH, 2xs, (aromatic C-H)-2″,6″), (**Figure 78**); *m/z* (MALDI-TOF) 553.6 $[MH]^+$

1-phenyl-2-(3′-(2″,4″,5″-trichlorophenoxypropyloxy)phenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-36)**

0.4058 g, yield 73 % (1.0 mmol scale); δ_H (DMSO, 200 MHz); 2.17 (2H, quintet, -$CH_2C\underline{H}_2CH_2$-), 4.09 (2H, t, *J*=7.0 Hz, -$OC\underline{H}_2$-), 4.24 (2H, t, *J*=7 Hz, -$C\underline{H}_2O$-), 6.02 (1H, s, H-2), 6.91 (2H, m, aromatic C-H), 7.16 (2H, d, *J*=8.0 Hz aromatic C-H), 7.17-7.44 (5H, m, aromatic C-H), 7.49 and 7.83 (2xH, 2xs, aromatic C-H)-2″,6″), (**Figure 79**); *m/z* (MALDI-TOF) 518.0 $[MH]^+$

1-(3′-chlorophenyl)-2-(3′-(2″,4″,5″-trichlorophenoxypropyloxy)phenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-37)**

0.0484 g, yield 16 % (0.5 mmol scale); FOUND C, 48.89; H, 3.58; N, 11.81 % ($C_{24}H_{22}Cl_5N_5O_2$ requires C, 48.88; H, 3.76; N, 11.88 %); δ_H (DMSO, 200 MHz); 2.17 (2H, quintet, -$CH_2C\underline{H}_2CH_2$-), 4.10 (2H, t, *J*=7.0 Hz, -$OC\underline{H}_2$-), 4.24 (2H, t, *J*=7.0 Hz, -$C\underline{H}_2O$-), 6.03 (1H, s, H-2), 6.90 (3H, m, aromatic C-H), 7.13 (1H, m, aromatic CH), 7.31-7.39 (4H, m, aromatic C-H), 7.50 and 7.80 (2xH, 2xs, (aromatic C-H)-2″,6″), (**Figure 80**); *m/z* (MALDI-TOF) 553.3 $[MH]^+$

1-(4′-chlorophenyl)-2(3′-(2″,4″,5″-trichlorophenoxypropyloxy)phenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-38)**

0.1448 g, yield 48 % (0.5 mmol scale); FOUND C, 48.87; H, 3.57; N, 11.84 % ($C_{24}H_{22}Cl_5N_5O_2$ requires C, 48.88; H, 3.76; N, 11.88 %); δ_H (DMSO, 200 MHz); 2.17 (2H, m, -$CH_2C\underline{H}_2CH_2$-), 4.10 (2H, t, *J*=7.0 Hz, -$OC\underline{H}_2$-), 4.25 (2H, t, *J*=7.0 Hz, -$C\underline{H}_2O$-), 5.98 (1H, s, H-2), 6.90 (2H, m, aromatic C-H), 7.30 (2H, d, aromatic C-H),

7.20 and 7.44 (2x2H, AB doublet, *J*=8.0 Hz, (aromatic C-H)-1), 7.50 and 7.80 (2xH, 2xs, (aromatic C-H)-2′′,6′′), (**Figure 81**); *m/z* (MALDI-TOF) 551.3 and 553.3 ($M.H^+$)

1-phenyl-2-(3′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-39)**

0.2629 g, yield 66 % (1.0 mmol scale); δ_H (DMSO, 200 MHz); 6.05 (1H, d, *J*=2.0 Hz, H-2), 6.89 (3H, m, aromatic C-H), 7.11 (1H, m, aromatic C-H), 7.14 (4H, m, aromatic C-H), 7.38 (6H, m, aromatic C-H), (**Figure 82**); *m/z* (MALDI-TOF) 358.2 $[MH]^+$

1-(4′-methylphenyl)-2-(3′′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-40)**

0.1544 g, yield 38 % (1.0 mmol scale); δ_H (DMSO, 200 MHz); 2.33 (3H, s, CH_3-4), 6.00 (1H, d *J*=2.0 Hz, H-2), 6.90 (3H, m, aromatic C-H), 7.02 (3H, m, aromatic C-H), 7.19 (4H, m, aromatic C-H), 7.45 (3H, m, aromatic C-H), (**Figure 83**); *m/z* (MALDI-TOF) 372.3 $[MH]^+$

1-(4′-chlorophenyl)-2-(3′′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-41)**

0.2841 g, yield 67 % (1.0 mmol scale); δ_H (DMSO, 200 MHz); 6.05 (1H, s, H-2), 6.88 (3H, m, aromatic C-H), 6.99 (1H, m, aromatic C-H), 7.17 (4H, m, aromatic C-H), 7.34-7.50 (5H, m, aromatic C-H), (**Figure 84**); *m/z* (MALDI-TOF) 391.7 $[MH]^+$

1-(4′-bromophenyl)-2-(3′′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-42)**

0.2960 g, yield 52 % (1.2 mmol scale); δ_H (DMSO, 200 MHz); 6.06 (1H, s, H-2), 6.88 (3H, m, aromatic C-H), 7.01 (1H, m, aromatic C-H), 7.04 and 7.09 (2x2H, 2xd, *J*=8.0 Hz, aromatic C-H), 7.42 (3H, m, aromatic C-H), 7.62 (2H, m, aromatic C-H), (**Figure 85**); *m/z* (MALDI-TOF) 435.8 and 437.8 $[MH]^+$

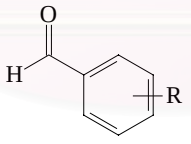
1-(4′-ethylphenyl)-2-(3′′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-43)**

0.2522 g, yield 73 % (0.8 mmol scale); δ_H (DMSO, 200 MHz); 1.15 (3H, t, *J*=7.0 Hz, -$CH_2C\underline{H}_3$-4′), 2.57 (2H, q, *J*=7.0 Hz, -$C\underline{H}_2CH_3$-4′), 6.01 (1H, d, *J*=2 Hz, H-2), 6.88 (3H, m, aromatic C-H), 7.04 (3H, m, aromatic C-H), 7.14 (2H, m, aromatic C-H), 7.24 (2H, d, *J*=8.0 Hz, aromatic C-H), 7.37 (3H, m, aromatic C-H), (**Figure 86**); *m/z* (MALDI-TOF) 386.96 $[MH]^+$

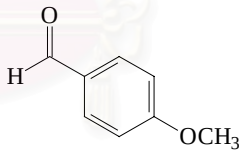
1-(3′,4′-dichlorophenyl)-2-(3′′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-44)**

0.1894 g, yield 41 % (1.0 mmol scale); δ_H (DMSO, 200 MHz); 6.09 (1H, d, H-2), 6.86 (2H, m, aromatic C-H), 7.12 (4H, m, aromatic C-H), 7.34 (4H, m, aromatic C-H), 7.55 and 7.65 (2xH, 2xd, *J*=8.0 Hz, aromatic C-H), (**Figure 87**); *m/z* (MALDI-TOF) 425.5 $[MH]^+$

2.9 Synthesis of substituted benzaldehyde derivatives



8a) R = 4-OBn
8b) R = 3-OBn



(9)

Typical procedure for synthesis of alkyloxybenzaldehyde

The mixture of hydroxybenzaldehyde (13-15 mmol), anhydrous potassium carbonate (14-18 mmol), acetonitrile (20-30 mL) and benzyl bromide or methyl-4-toluene sulphonate (14-18 mmol) were heated under reflux for 5-6 hr until the starting material disappeared according toTLC analysis. The mixture was evaporated, then water was added to the residue. The mixture was extracted with CH_2Cl_2 and the organic layer was washed with 10 % aqueous NaOH to remove the excess hydroxybenzaldehyde. After evaporation of the organic layer, the pure product was obtained.

4-benzyloxybenzaldehyde **(8a)**

3.4024 g, yield 100 % (15.0 mmol scale); δ_H ($CDCl_3$, 200 MHz); 5.14 (2H, s, C<u>H</u>$_2$Ph), 7.08 and 7.84 (2x2H, AB doublet, *J*=8.0 Hz, aromatic C-H), 7.34-7.45 (5H, m, aromatic C-H), 9.88 (1H, s, H- aldehyde)

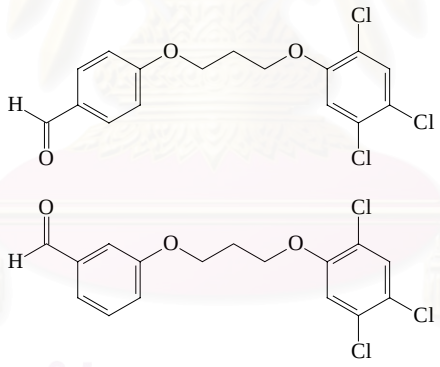
3-benzyloxybenzaldehyde **(8b)**

3.1845 g, yield 100 % (15.0 mmol scale); δ_H ($CDCl_3$, 200 MHz); 5.20 (2H, s, C<u>H</u>$_2$Ph), 7.22-7.47 (9H, m, 2x aromatic C-H), 9.96 (1H, s, H- aldehyde)

4-methoxybenzaldehyde **(9)**

1.8581 g, yield 91 % (13.5 mmol scale); δ_H ($CDCl_3$, 200 MHz); 3.85 (3H, s, -OCH_3), 6.98 and 7.80 (2x2H, AB doublet, *J*=8.0 Hz, aromatic C-H), 9.85 (1H, s, H-aldehyde)

<u>Typical procedure for synthesis of 2′,4′,5′-trichlorophenoxy propyloxybenzaldehydes</u>



(10a)

(10b)

Hydroxybenzaldehyde (11 mmol) was added to a mixture of 2,4,5-trichloro-phenoxypropylbromide (10 mmol) and anhydrous potassium carbonate (11 mmol) in acetonitrile (10 mL). The reaction mixture was heated under reflux until the starting materials disappeared according to TLC analysis (6-7 hrs). The solvent was evaporated and the residue was extracted with CH_2Cl_2. The extracted organic was washed (10 % NaOH), dried ($MgSO_4$) and evaporated to give the crude product which is sufficiently pure for practical purpose.

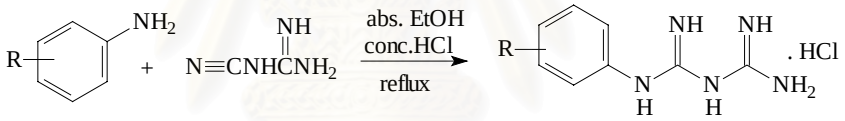
4-(2′,4′,5′-trichlorophenoxypropyloxy)benzaldehyde **(10a)**

3.2149 g, yield 89 % (10.0 mmol scale); δ_H ($CDCl_3$, 200 MHz); 2.32 (2H, quintet, -$CH_2C\underline{H}_2CH_2$-), 4.19 (2H, t, *J*=7.5 Hz, -CH_2O-), 4.27 (2H, t, *J*=7.5 Hz, -OCH_2-), 7.00 and 7.42 (2xH, 2xs, aromatic C-H), 7.02 and 7.82 (2x2H, AB doublet, *J*=8.0 Hz, aromatic C-H), 9.87 (1H, s, H-aldehyde)

3-(2′,4′,5′-trichlorophenoxypropyloxy)benzaldehyde **(10b)**

3.1572 g, yield 89 % (9.8 mmol scale); δ_H ($CDCl_3$, 200 MHz); 2.32 (2H, quintet,-$CH_2C\underline{H}_2CH_2$-), 4.20 (2H, t, *J*=7.5 Hz, -CH_2O-), 4.22 (2H, t, *J*=7.5 Hz, -OCH_2-), 7.00 and 7.38 (2xH, 2xs, aromatic C-H), 7.18 and 7.45 (4H, 2xm, aromatic C-H), 9.95 (1H, s, H-aldehyde)

2.10 Synthesis of arylbiguanide hydrochloride



(1)

a)	R= 4-Cl	**h)**	R= H
b)	R= 4-Et	**i)**	R= 4-Me
c)	R= 4-Br	**j)**	R= 3,4-Cl_2

Typical procedure for synthesis of arylbiguanide hydrochloride

A solution of the arylamine (25 mmol) in absolute ethanol (10 mL) containing conc.HCl (27.5 mmol) and dicyanodiamide (27.5 mmol) was heated to reflux (6-10 h). After cooling in the fridge for several hours, the colorless crystalline precipitate was collected by filtration and washed with EtOH, acetone, Et_2O and then air dried.

4-chlorophenylbiguanide hydrochloride **(1a)** 4.5914 g, yield 75 % (24.7 mmol scale), purified by recrystallization from EtOH and water, obtained as colorless needles; δ_H (D_2O, 200 MHz); 7.06 and 7.22 (2x2H, AB doublet, *J*=8.0 Hz, aromatic C-H)

4-ethylphenylbiguanide hydrochloride **(1b)** 2.5679 g, yield 41 % (25.6 mmol scale), purified by recrystallization from EtOH and water, obtained as colorless prisms; δ_H (D_2O, 200 MHz); 0.98 (3H, t, *J*=7.0 Hz, $CH_2C\underline{H}_3$), 2.44 (2H, q, *J*=7.0 Hz, $C\underline{H}_2CH_3$), 7.00 and 7.11 (2x2H, AB doublet, *J*=8.0 Hz, aromatic C-H)

4-bromophenylbiguanide hydrochloride **(1c)** 5.6821 g, yield 78 % (25.0 mmol scale), purified by recrystallization from EtOH and water, obtained as colorless plates; δ_H (D_2O, 200 MHz); 7.00 and 7.37 (2x2H, AB doublet, *J*=8.0 Hz, aromatic C-H)

Phenylbiguanide hydrochloride **(1h)** 4.1235 g, yield 70 % (27.5 mmol scale), purified by recrystallization from EtOH and water, obtained as colorless prisms; δ_H (D_2O, 200 MHz); 7.11 and 7.24 (5H, 2xm, aromatic C-H)

4-methylphenylbiguanide hydrochloride **(1i)** 3.7995 g, yield 66 % (25.2 mmol scale), purified by recrystallization from EtOH and water, obtained as colorless prisms; δ_H (D_2O, 200 MHz); 2.12 (3H, s, $C\underline{H}_3$), 6.97 and 7.07 (2x2H, AB doublet, *J*= 8.0 Hz, aromatic C-H)

3,4-dichlorophenylbiguanide hydrochloride **(1j)** 3.8940 g, yield 53 % (26.2 mmol scal), purified by recrystallization from EtOH and water, obtained as colorless plates; δ_H (D_2O, 200 MHz); 6.97 (1H, dd, *J*=8.5, 2.0 Hz, aromatic C-H)

2.11 Enzyme assays and inhibition by cycloguanil analogues

The activities of wild-type and A16VS108T mutant pfDHFRs were determined spectrophotometrically according to the method previously described[8]. The reaction (200 μL) contained 1x DHFR buffer (50 mM TES, pH 7.0, 75 mM β-mercaptoethanol, 1 mg/mL Bovine Serum Albumin), 100 μM each of the substrate H_2folate and cofactor NADPH, and appropriate amount (0.001-0.005 units) of the affinity-purified enzymes. The inhibition of the enzymes with cycloguanil analogues and combinatorial libraries was investigated in a 96 well plate with 200 μL reaction of the above mixture, in the presence of antifolate. The kinetic reaction was followed by a microplate reader (Labsystems, Finland). The K_i values of the inhibitors for the enzymes were then determined by fitting to the equation $IC_{50} = K_i (1+([S]/K_m))$,[31]

where IC_{50} is the concentration of inhibitor which inhibits 50% of the enzyme activity under the standard assay condition and K_m is the Michaelis constant for the substrate H_2folate.

CHAPTER III

RESULTS AND DISCUSSION

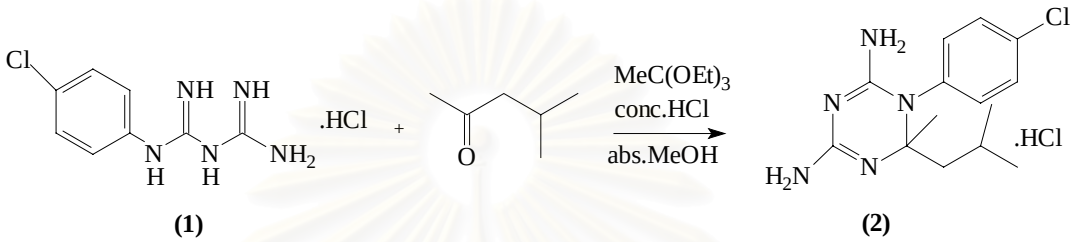
The objectives of this research were to develop a method for the synthesis of combinatorial libraries of 4,6-diamino-1,2-dihydro-1,3,5-triazine, and to identify and characterize the compound showing the best inhibition constants (K_i) against wild type and A16VS108T mutant DHFRs of *P. falciparum*.

Due to the fact that products from combinatorial synthesis are mixture, quality of combinatorial libraries were of supreme importance if we wish to obtain reliable inhibition constants for screening. Consequently optimizing the synthesis method to give combinatorial libraries of highest quality was necessary. The two-component synthesis method (**Scheme 1.7**) was selected as a starting point because this method was appropriate for the split synthesis technique. Previous work[32] has revealed that the improvement could be done by addition of triethyl orthoacetate (TEOA) as dehydrating agent. However, it was found difficult to obtain good yield of dihydrotriazine reproducibly. As a result, the first step of this research was optimization of reaction condition.

3.1 Optimization of reaction for synthesis of 4,6-diamino-1,2-dihydro-1,3,5-triazine

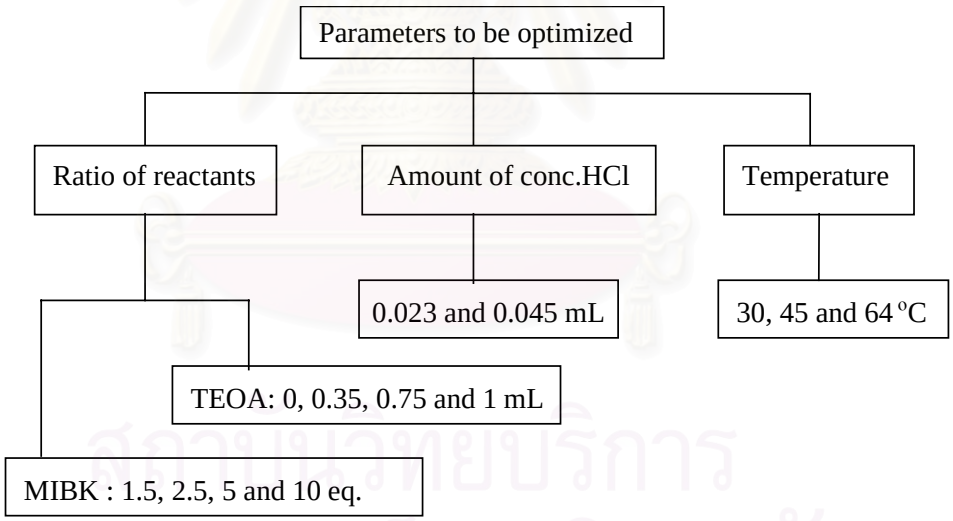
The purpose of this study was to optimize reaction condition using a reaction between 4-chlorophenylbiguanide hydrochloride and methyl isobutyl ketone (MIBK) as a model (**Figure 3.1)**. This model system was selected because MIBK is a relatively sterically hindered ketone and all attempts to make this compound by literature methods[28,29] failed to give the desired product. Three parameters were optimized, including the ratio of reactants, the amounts of acid catalyst and the temperature (**Scheme 3.1)**. Progress of the reaction was monitored by HPLC on a reverse-phase C_{18} column with a mobile phase consisting of a mixture of triethylammonium acetate buffer (0.1 M, pH 7.0) and acetonitrile. The composition of acetonitrile was changed linearly from 15 to 60 % during 15 min while the percentage

of the buffer used was altered accordingly. The buffer and gradient system used was chosen from a previous work in this group.[33] The HPLC chromatogram from a typical reaction mixture from reaction of 4-chlorophenylbiguanide hydrochloride, MIBK, HCl and TEAO revealed, in addition to the starting material **(1)** and 4,6-diamino-1,2-dihydro-1,3,5-triazine product **(2)**, two side products **(3)** and **(4)**. All compounds were readily identified by λ_{max} and retention time (t_R) of each peak **(Figure 3.2**).

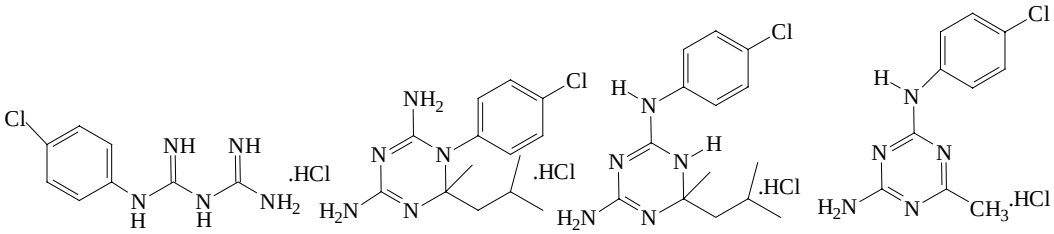


(1) **(2)**

Figure 3.1: The model reaction used for optimization of the synthesis.



Scheme 3.1: Three parameters for optimization of reaction



(1) **(2)** **(3)** **(4)**

λ_{max} (nm)	253.7	244.3	263.2	244.3
t_R (min.)	9.3	15.5	16.2	17.5

Figure 3.2: Structure, λ_{max} and t_R of **(1)**, **(2)**, **(3)** and **(4)**

The peak area of each compound from the HPLC chromatogram was converted to % peak area by division with total peak areas. The optimized reaction condition was indicated by examination of % peak area of **(2)**. The first parameter to be optimized was the reactant ratio. This is first attempted by varying the amount of MIBK to 1.5, 2.5, 5 and 10 eq, while the scale of reactions and temperature remained constant at 1 mmol and 45 °C respectively. The results (**Figure 3.3**) indicated that the conversion is more complete at higher amount of MIBK used. The reaction was 43 % complete at 1.5 and 2.5 eq of MIBK after 12 hrs at 45 °C. Under the same condition at 5 and 10 eq of MIBK, the reaction was 71 and 73 % complete. When MIBK was used in less than 5 mmol, **(2)** was produced in less than 50 %. It is evidenced that if MIBK was used more than 5 mmol, **(2)** would still be formed in nearly the same amount as when MIBK 5 eq was used. Therefore, 5 equivalents of MIBK were taken as the optimized amount of MIBK and used for further experiments.

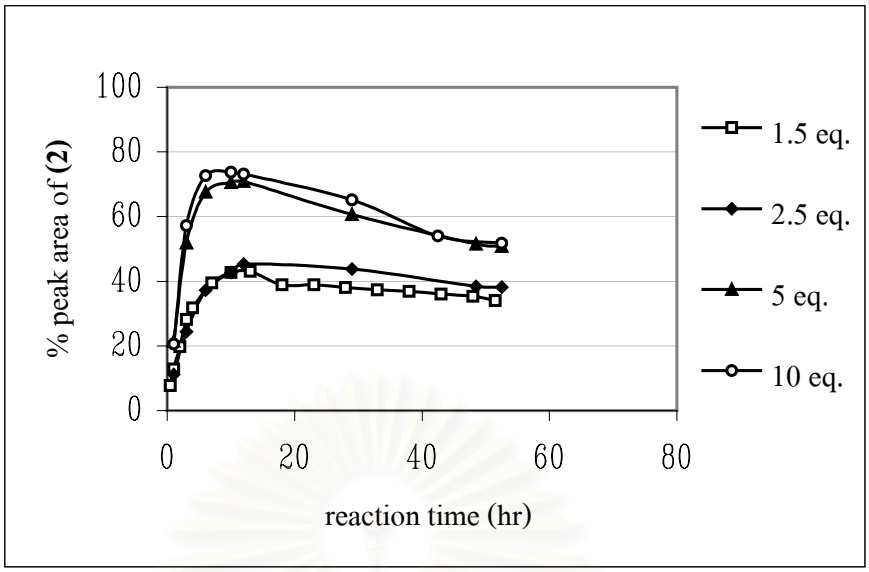


Figure 3.3: % Peak areas of **(2)** from HPLC analysis of varying amounts of MIBK: (4-ClC_6H_4biguanide (1 mmol), TEOA 0.61 mL, HCl 0.045 mL (2 drops), T = 45 ^{o} C)

In the next experiment, the amount of triethyl orthoacetate (water scavenger) was varied at 0, 0.35, 0.75 and 1 mL. The results were presented in **Table 2.5-2.8** and **Figure 3.4**. From the results, TEOA 0.75 and 1 mL could generate the maximum % peak areas of **(2)** at 76 and 79 % respectively within 4 hr. While at TEOA at 0.35 mL, **(2)** was generated in less than 40 % after 63 hr. It is clear that addition of triethyl orthoacetate considerably reduce the time to give maximum amounts of product and addition of more TEOA could increase the yield of **(2)**. This can be explained by the fact that condensation reaction to form **(2)** produce water (**Scheme 3.2**), and TEOA acted as dehydrating agent which can shift the equilibrium to the right. The mechanism of water removal from the reaction could be proposed as shown in **Scheme 3.3**.

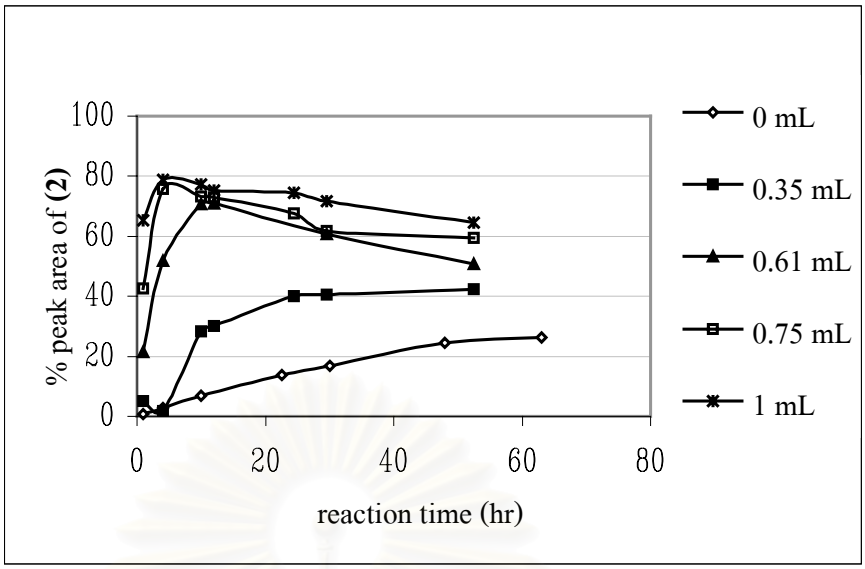
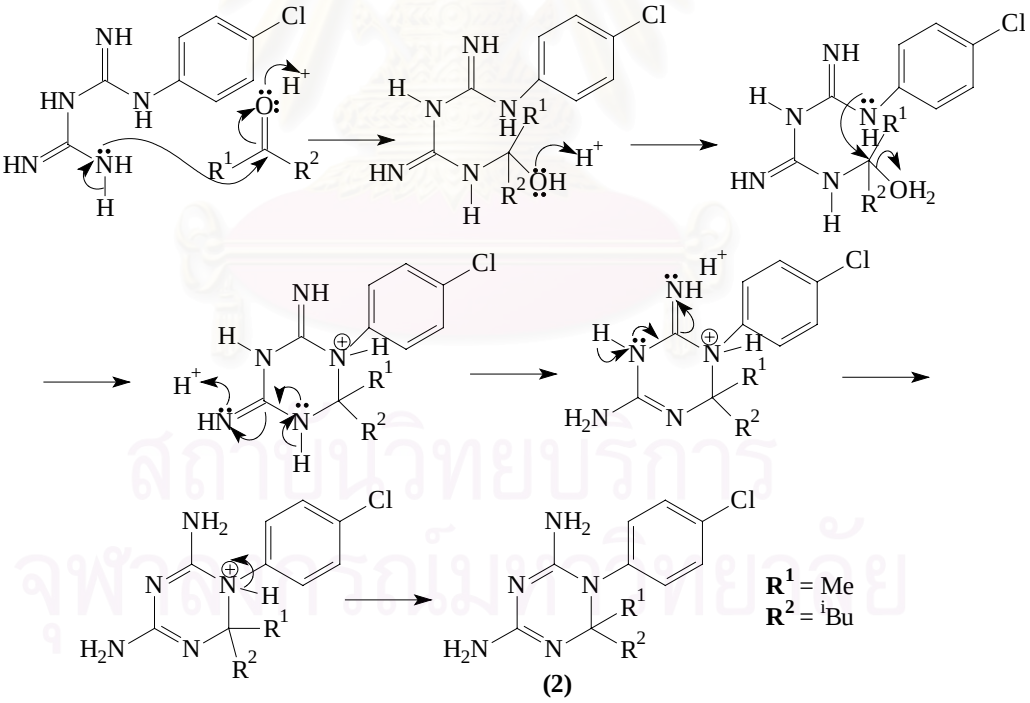
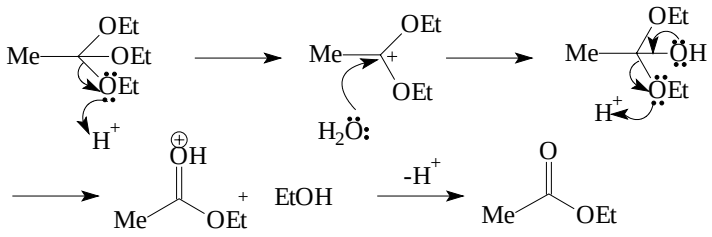


Figure 3.4: % Peak areas of **(2)** from HPLC analysis of varying amounts of TEOA: (4-ClC_6H_4biguanide (1 mmol), MIBK 5 eq, HCl 0.045 mL (2 drops), T = 45 ^{o} C)

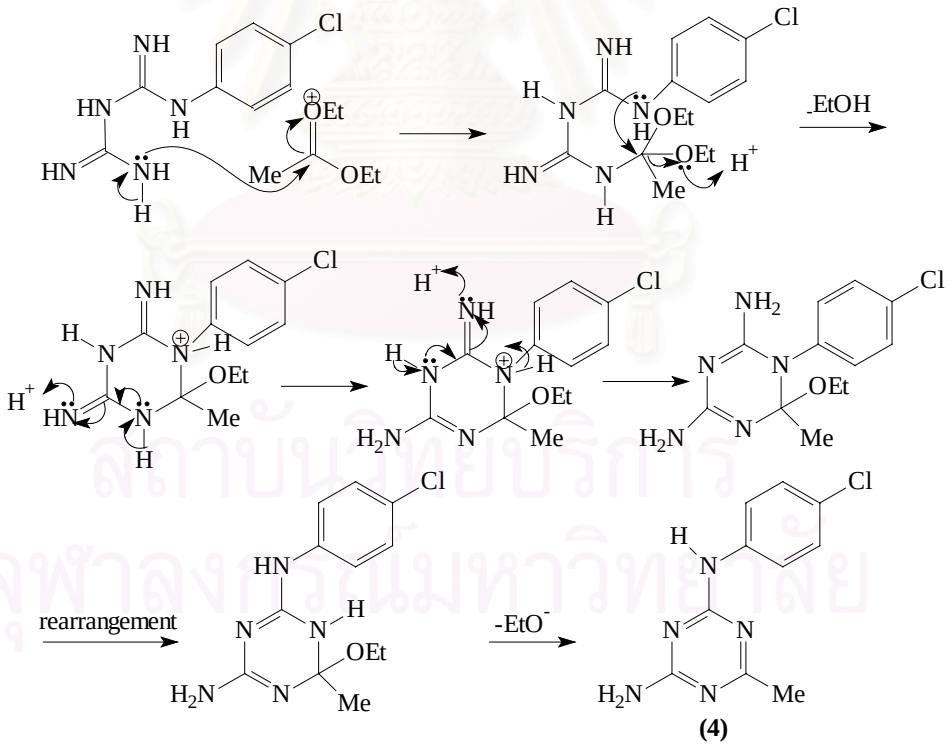


Scheme 3.2: The reaction mechanism of biguanide and ketone to form **(2)**



Scheme 3.3: A proposed mechanism of TEOA as dehydrating agent

However, the disadvantage of using TEOA was the formation of by-product **(4)**. The presence of **(4)** was evidenced in all reaction involving TEOA as shown by the peak at t_R = 17.5 min and λ_{max} = 244.3 nm. The mechanism for the formation of **(4)** was proposed in **Scheme 3.4**. This probably accounts for the decrease in the yield of the product after a long reaction time when high concentration of TEOA was used. Therefore, the amount of TEOA at 0.75 mL/mmol was chosen as the optimized amount of TEOA for further experiments.



Scheme 3.4: A proposed reaction mechanism of **(4)**

The second parameter to be varied in the optimization of reaction conditions was the amount of the acid catalyst. Preliminary experiments suggested that concentrated HCl is the most efficient catalyst therefore conc. HCl was used as the acid catalyst in the optimization process, keeping the amount of ketone and TEOA constant at 5 eq and 0.75 mL/mmol respectively. The amount of conc.HCl were varied between 0.045 mL (2 drops) and 0.023 mL (1 drop). The results were as shown in **Table 2.7** and **2.9** and **Figure 3.5**. The results showed that 0.023 mL of conc.HCl produce better yield of **(2)** than when 0.045 mL of conc.HCl. This is probably because HCl was used as aqueous solution, so if the volume of conc.HCl was increased, the amount of water present in the reaction would be increased too. This increased water may give rise to the hydrolysis of the condensation product. As a result, the volume of at conc.HCl at 0.023 mL was selected as the most appropriate volume to be used in further optimization of the reaction condition.

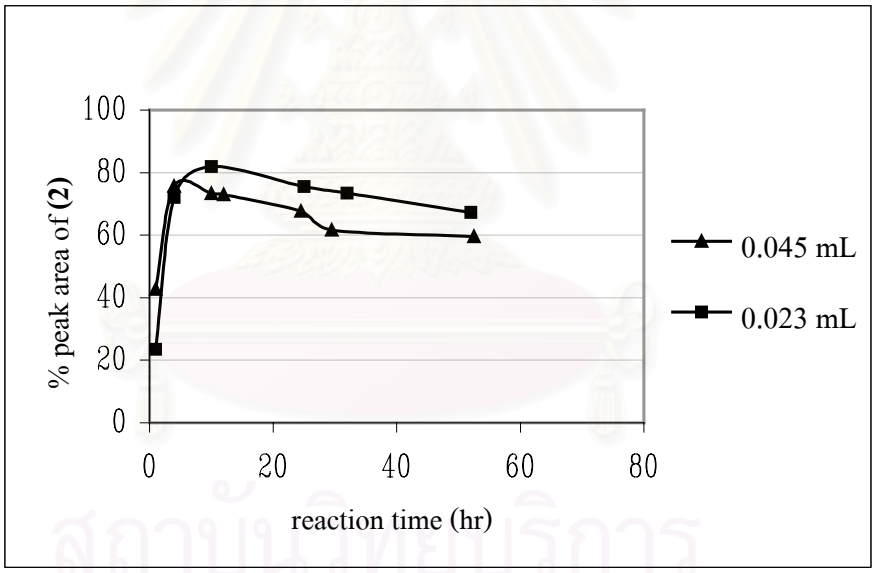


Figure 3.5: % Peak areas of **(2)** from HPLC analysis of varying amounts of conc.HCl: (4-ClC_6H_4biguanide (1 mmol), MIBK 5 eq, TEOA 0.75 mL, T = 45 ^{o} C)

The last parameter to be optimized was reaction temperature. In this study, the temperature was set at 45 $^{\circ}$C (in an oil bath), 30 $^{\circ}$C (room temperature, rt) and 64 $^{\circ}$C (reflux). The results were as shown in **Table 2.9**, **2.10** and **2.11** and **Figure 3.6**. At reaction temperature of 30 $^{\circ}$C, % peak area of **(2)** was slowly increased to 80 %

during a period of 12 hr and remained more or less and remained constant even after the reaction time as long as 49 hr. At reflux temperature, % peak area of **(2)** was rapidly increased to 70 % during in the first hour of the reaction but it was decreased to 39 % during the next 7 hours. At reaction temperature of 45 °C, % peak area of **(2)** was increased to 75 % at the 4th hour. After that the rate of decreasing of % peak area of **(2)** was higher than the reaction temperature at 30 °C. Consequently, the temperature of 30 °C was selected as the optimized reaction temperature.

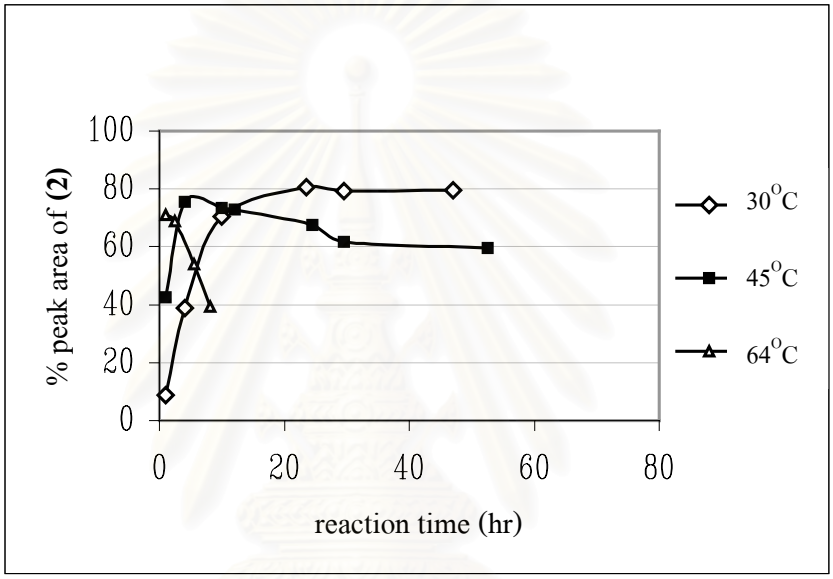


Figure 3.6: % Peak areas of **(2)** from HPLC analysis of varying temperature: (4-ClC_6H_4biguanide (1 mmol), MIBK 5 eq, TEOA 0.75 mL, conc.HCl = 0.023 mL)

In conclusion, the optimized reaction condition for the synthesis at 1 mmol scale of 4-chlorophenylbiguanide hydrochloride was found to be: 5 mmol of MIBK, 0.75 mL of TEOA and 0.023 mL of conc.HCl and the reaction performed at 30 °C. The HPLC chromatograms of the reaction mixture before and after optimization, were compared as shown in **Figure 3.7**.

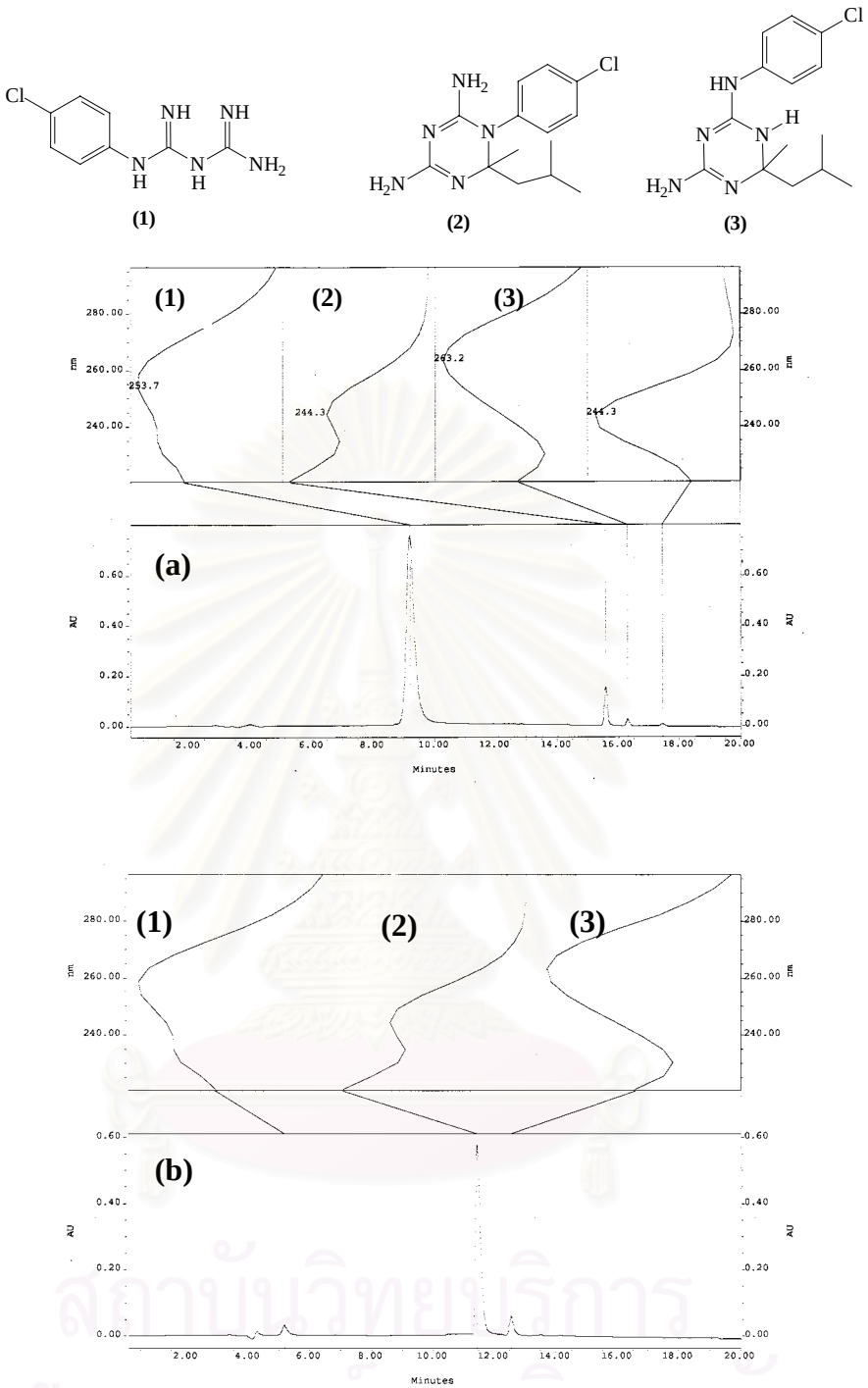


Figure 3.7: (a) HPLC chromatogram of the reaction before optimization (conditions: 1 mmol of 4-chlorophenylbiguanide hydrochloride, 5 mmol of MIBK, 0.023 mL of conc.HCl, TEOA = 0 mL, 45 °C, at 22.5 hr) **(b)** HPLC chromatogram of the reaction mixture after optimization (conditions: 1 mmol of 4-chlorophenylbiguanide hydrochloride, 5 mmol of MIBK, 0.023 mL of conc.HCl, TEOA = 0.75 mL, 30 °C at 22.5 hr)

3.2 The effect of base and acid to the rearrangement of 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazine (2) to 4-amino-6-anilino-1,2-dihydro-1,3,5-triazine (3)

Rearrangement from **(2)** to **(3)** was often observed during optimizations of the reaction condition and usually led to losing of DHFR inhibition activity. It was reported that rearrangement from **(2)** to **(3)** occurred under basic conditions[28]. The purpose of this study was to further investigate the effect of basicity and other effects such as acidity and temperature on the rearrangement of **(2)** to **(3)**. The result from this study would be beneficial in controlling the quality of the combinatorial libraries being synthesized. The experiment was set up as shown in **Figure 3.8**.

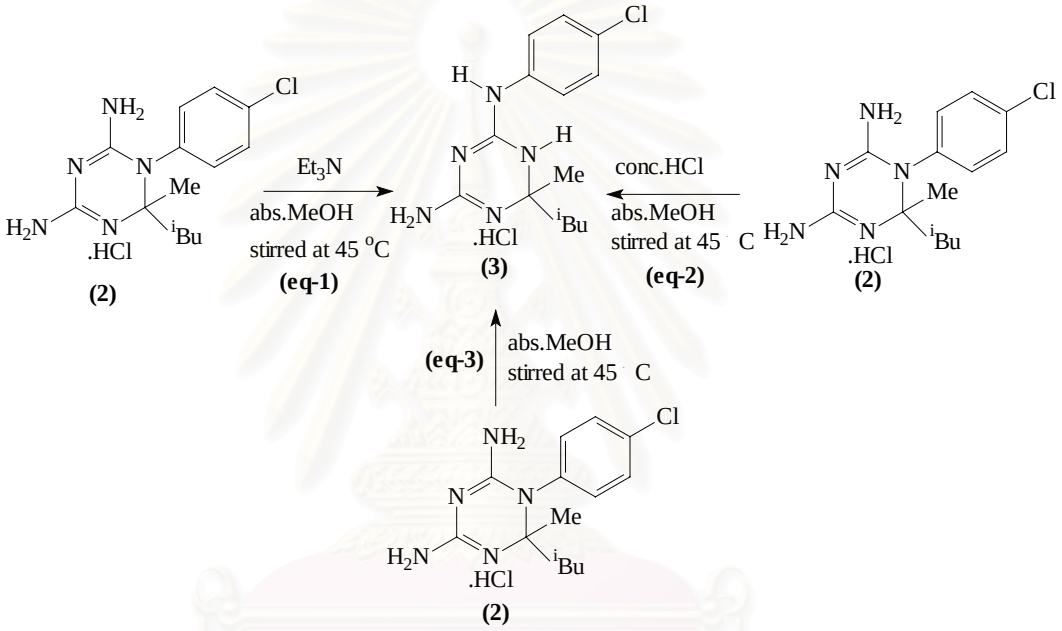
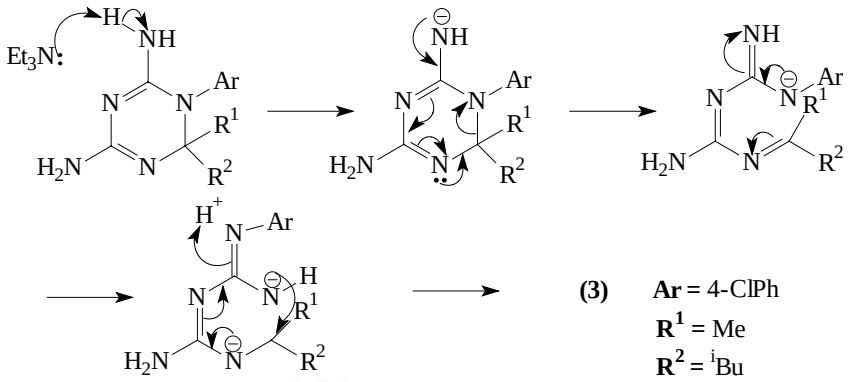


Figure 3.8: Three reactions were set-up to study the rearrangement of **(2)** to **(3)**

All reactions were monitored by HPLC. The results revealed that the rearrangement from **(2)** to **(3)** was fastest in the presence of triethylamine, as previously reported. The base-catalyzed rearrangement mechanism[28] has been proposed as shown in **Scheme 3.5**. Rearrangement of **(2)** under acidic condition has not been previously reported. However, when **(2)** was treated with conc.HCl at 45 °C in order to study the effect of acidity in the rearrangement reaction, hydrolysis of **(2)** to 4-chlorophenylbiguanide **(1)**, was observed 85 % together with 5 % of the rearrangement product **(3)** after a reaction time of 2 hours. On increasing the reaction time to 44 hours, % peak area of **(1)** was decreased to 70 % and % peak area of **(3)** was increased to 28 % (**Figure 3.9**).



Scheme 3.5: The mechanism of rearrangement from **(2)** to **(3)** with Et_3N[28]

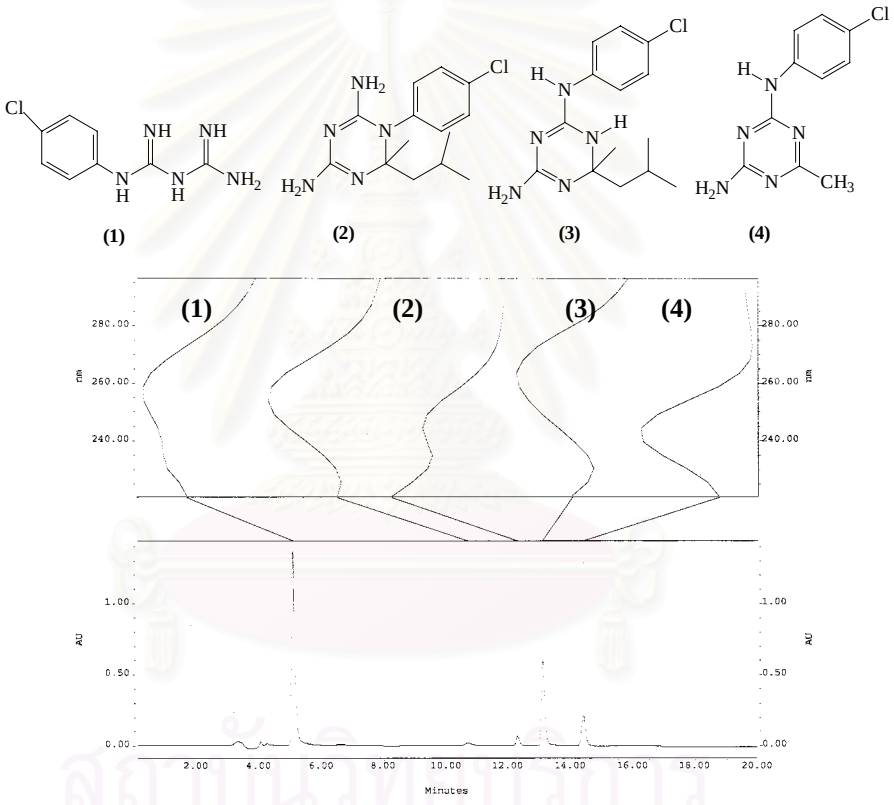
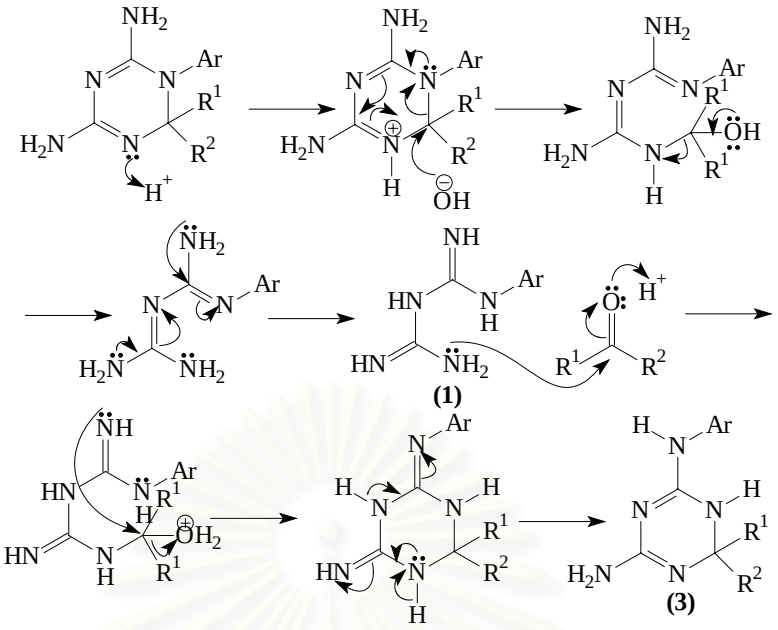


Figure 3.9: The result from HPLC analysis of **(eq-2)** at reaction time of 44 hr.

The results observed above are consistent with the 2-step rearrangement mechanism. The first step involved hydrolysis **(2)** to **(1)** and then in the second step **(3)** was formed by recyclization of **(1)**. As **(3)** would be the thermodynamically more stable product, it should form better under equilibrium conditions. The mechanism was proposed as shown in **Scheme 3.6**.



Scheme 3.6: The proposed mechanism of rearrangement from **(2)** to **(3)** under acidic condition

In a controlled reaction, neither Et_3N nor HCl was added, up to 50 % of the rearrangement from **(2)** to **(3)** was observed at the reaction time of 44 hours with the absence of **(1)** (**Figure 3.10**). This rearrangement had the mechanism like **Scheme 3.5** but methanol itself probably act as a base to attack the proton. The results revealed that **(2)** may rearrange to **(3)** under basic, acidic and neutral condition. The rate of rearrangement was fastest under basic and slowest under neutral conditions.

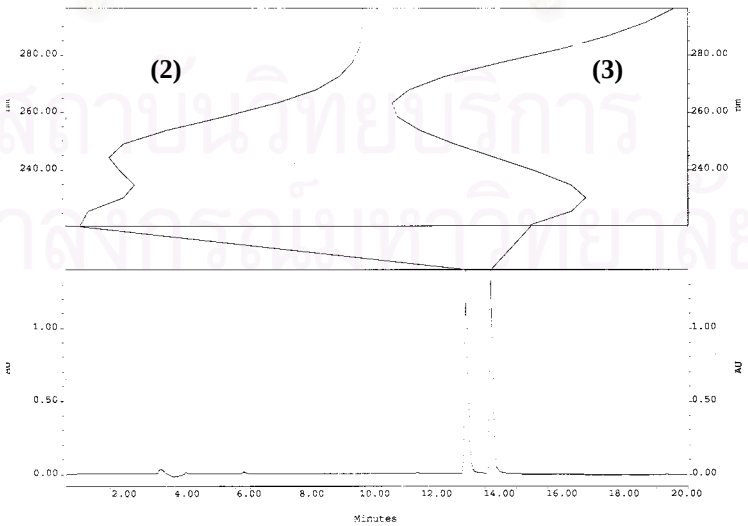
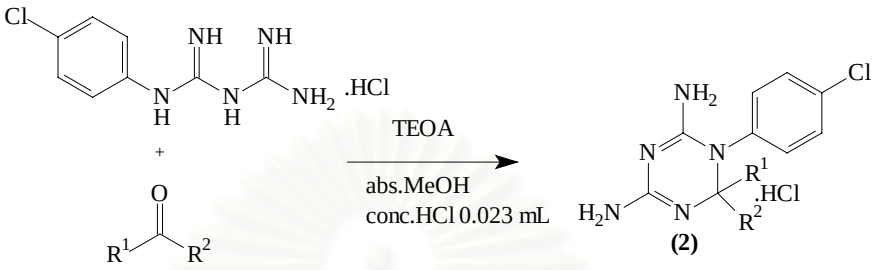


Figure 3.10: The result of **(eq-3)** from HPLC analysis at a reaction time of 44 hr

3.3 Study of structure of carbonyl compounds and substituent on benzene ring in synthesis of 4,6-diamino-1,2-dihydro-1,3,5-triazine

3.3.1 The effect of structure of carbonyl compounds



Carbonyl compounds used in this study were chosen from those bearing substituents with different steric effect including benzaldehyde, acetone, 4-heptanone, MIBK and 3-methyl-2-butanone. The rate of reaction was qualitatively determined from the slope of % peak area of **(2)** against time, as analyzed by HPLC. The results indicated that the sequence of carbonyl compound, sorted according to their reaction rate from fastest to slowest were MIBK, acetone, 4-heptanone, 3-octanone, benzaldehyde and 3-methyl-2-butanone (**Figure 3.11**). This sequence was consistent with the structure of the carbonyl compounds based on their steric ground except benzaldehyde and acetone. This can be explained that both compounds may react with absolute methanol (solvent in reaction) to give acetals leading to lower effective concentration compared to other carbonyl compounds. Therefore, benzaldehyde and acetone, which were least steric hindered did not give fastest reactions. However, it should be noted that under this optimized condition, all ketones tested (expect for the highly hindered 3-methyl-2-butanone) reacted more or less completely within 80 hours, regardless of their difference in steric effect.

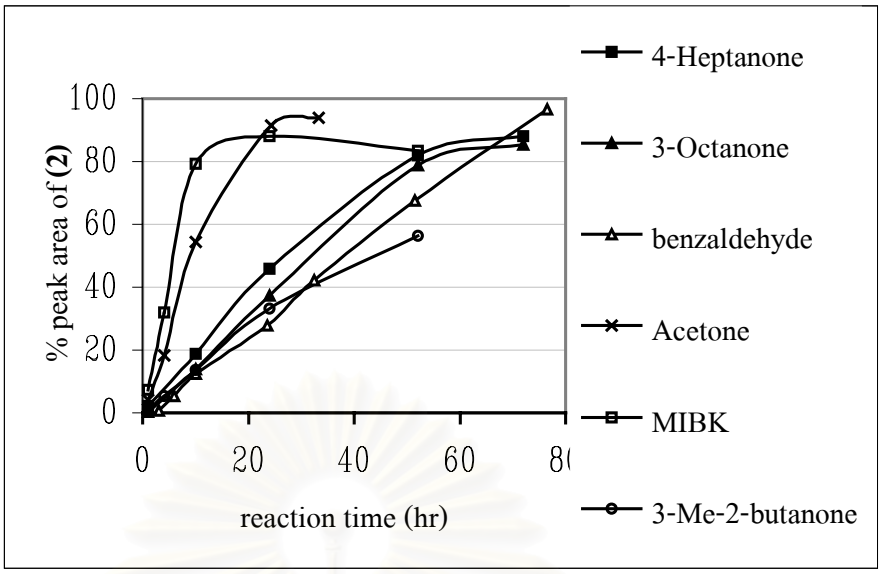
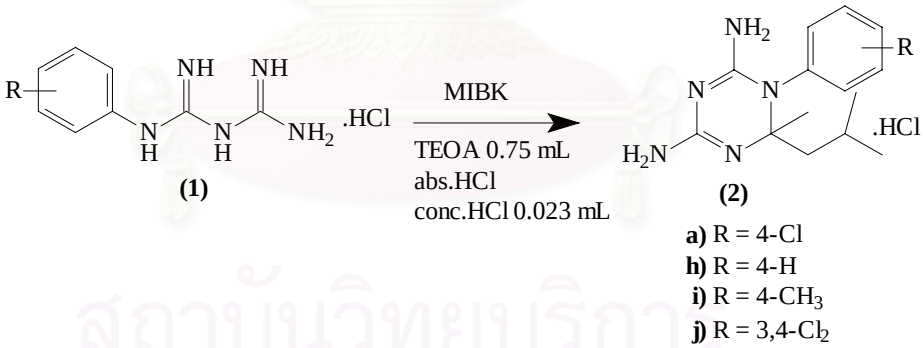


Figure 3.11: % peak area of **(2)** in study effect of sterically hindered carbonyl compounds to rate of reaction: (4-ClC_6H_4biguanide (1 mmol), carbonyl compound 5 eq, TEOA 0.75 mL, conc.HCl = 0.023 mL, T = 30 ^oC)

3.3.2 The effect of substitutents on benzene ring



The purpose of this study was to test the validity of applying combinatorial principle in the synthesis of dihydrotriazine using biguanides of different reactivities and also to study the electronic effect of the substituent on the benzene ring. 4-Methylphynylbiguanide hydrochloride and 3,4-chlorophenylbiguanides hydrochloride were used as representative biguanides bearing electron-donating and electron-withdrawing groups on the benzene ring respectively.

From **Figure 3.12**, it was found that the rate of formation of **(2j)** was the fastest and the reaction rate of **(2i)** was the slowest. However, during the study it was

observed that **(1j)** and **(1h)** do not dissolve well, therefore, care should be taken in interpreting the results. Nevertheless, the experiment has demonstrated that all biguanide react more of less completely within 50 hrs thus it should be possible to apply the combinatorial technique to synthesize library of dihydrotriazines bearing substituents on the N_1- benzene ring with different electron-donating or releasing power.

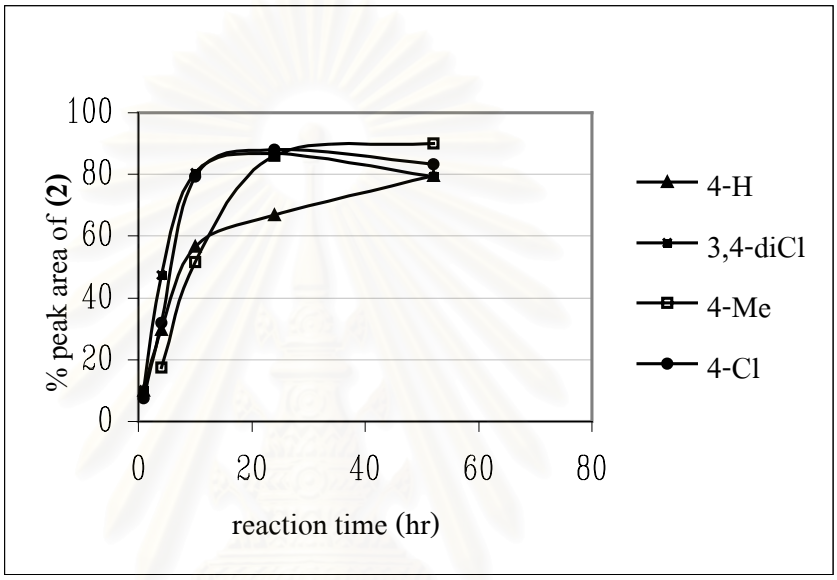
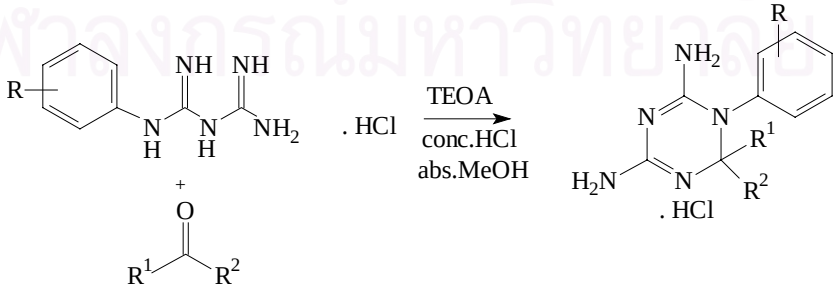


Figure 3.12: % peak area of **(2)** in study effect of substituted group on benzene ring to rate of reaction: (biguanide (1 mmol), MIBK 5 eq, TEOA 0.75 mL, conc.HCl = 0.023 mL, T = 30 ^{o}C)

3.4 Synthesis of individual 4,6-diamino-1,2-dihydro-1,3,5-triazine derivatives



To test the generality of the developed method, a number of dihydrotriazine have been synthesized (**Table 3.1**). We are pleased to find that fair to good yield of

the products (38-85 %) were obtained in all cases even with the sterically hindered ketones **(2-4)** to **(2-18)**. All compounds showed expected ^{1}HNMR and MALDI-TOF mass spectra, and most showed expected combustion analysis (C, H, N).

Table 3.1: Reaction time, % Yield (crude) and Mass Spectrum of synthesized derivatives of 4,6-diamino-1,2-dihydro-1,3,5-triazine by developed method in this research

(2)	R^1	R^2	R	reaction time (hr)	%yield (crude)	m/z	
						$[MH]^+_{obs.}$	$[MH]^+_{cal.}$
1	Me	Me	4-Cl	73	98	252.4	252.1
2	Me	Pr^n	4-H	49	93	246.2	246.2
3	Me	Pr^n	4-Me	49	82	260.5	260.2
4	Et	Et	4-H	140	73	246.2	246.2
5	Et	Et	$3,4\text{-}Cl_2$	139	88	314.0	315.2
6	$-(CH_2)_4-$		4-H	120	61	244.1	244.2
7	$-(CH_2)_4-$		4-Me	120	78	258.1	258.2
8	Me	Pr^i	4-Et	75	59	275.5	274.2
9	Me	Pr^i	4-H	7 day	67	246.0	246.2
10	Me	Pr^i	4-Cl	75	38	281.4	280.8
11	Me	Pr^i	$3,4\text{-}Cl_2$	75	84	315.6	314.1
12	Me	Bu^i	4-Me	52	75	275.8	274.2
13	Me	Bu^i	4-Cl	24	62	295.6	294.1
14	Me	Bu^i	$3,4\text{-}Cl_2$	24	53	328.1	328.0
15	Me	$(CH_3)_2CH(CH_2)_2-$	$3,4\text{-}Cl_2$	139	75	341.9	342.1
16	Pr^n	Pr^n	4-Cl	72	68	308.2	308.2
17	Me	Pen^n	$3,4\text{-}Cl_2$	42	91	342.1	341.8
18	Et	Pen^n	4-H	140	79	288.5	288.2
19	Et	Pen^n	4-Cl	72	64	324.1	321.2
20	Me	Hex^n	4-H	92	89	288.5	288.2
21	Me	Hex^n	4-Me	45	90	303.7	302.2
22	Me	Hex^n	4-Et	75	84	316.5	316.3
23	Me	Hex^n	4-Cl	75	85	322.5	322.2
24	Me	Hex^n	4-Br	45	86	366.1, 368.1	366.1, 368.1

Table 3.1: (continued)

(2)	R^1	R^2	R	reaction time (hr)	%yield (crude)	m/z	
						$[MH]^+_{obs.}$	$[MH]^+_{cal.}$
25	Me	Hex^n	3,4-Cl_2	42	92	356.4	356.1
26	H	Ph	4-Cl	76.5	69	300.4	299.1
27	H	4-$PhCH_2OC_6H_4$	4-Et	45	93[a]	400.1	400.2
28	H	4-$PhCH_2OC_6H_4$	4-Br	45	75[a]	449.9, 452.0	450.1, 452.1
29	H	4-$PhCH_2OC_6H_4$	4-H	48	55[a]	372.6	372.2
30	H	4-$PhCH_2OC_6H_4$	4-Me	48	73[a]	385.7	386.2
31	H	4-$PhCH_2OC_6H_4$	4-Cl	45	75[a]	405.7	406.1
32	H	4-$PhCH_2OC_6H_4$	3,4-Cl_2	45	64[a]	439.8	440.1

[a] % yield of crystalline product.

3.5 Synthesis of combinatorial libraries of 4,6-diamino-1,2-dihydro-1,3,5-triazine

In 1999, combinatorial libraries of 72 dihydrophenyltriazine compounds having *ortho*-, *para*- or *meta*-halogen, methyl, methoxy or nitro substitution on the phenyl ring and with 2,2-dimethyl, 2,2-cyclopentylidene or 2,2-cyclohexylidene substitution at the C-2 position of dihydrotriazine ring were synthesized by Hong-Kee Lee and Wai-Keung Chui.[34] The one-pot three component synthesis was employed. From the results of screening of all sub-libraries, it was found that dihydrotriazine having 2,2-dimethyl or 2,2-cyclopentylidene substitution at the C-2 position showed inhibitory action against rat liver DHFR. However, only 64 out of the expected 72 compounds were detected by HPLC analysis. We suspected that this was due to the difference of solubility of each dihydrophenyltriazine product. As the method relies on precipitation of crystalline dihydrotriazine from the reaction mixture, it is unlikely to expect that all the product would precipitate from the solution in equimolar quantities. Furthermore, each starting material reacted at different rate, therefore it is impossible to control the ratio of the product. The objective of this research was to develop an improved method to synthesize combinatorial libraries of 4,6-diamino-1,2-dihydro-1,3,5-triazine which would generate combinatorial library containing more diverse compounds, each presenting equimolar quantities regardless of relative

solubility of the products and reactivities of the starting materials. This obviously could not be accomplished by conventional methods but was considered possible with the help of the newly developed method. The library synthesized was designed as a solution-phase mixture library for screening using iterative deconvolution approach. Each sub-library in this research was synthesized by keeping one component unchanged while the other component varied. Ketone was the excess component used in our developed two-component synthesis of dihydrotriazine because it can be removed by evaporation and/ or washing with ether. As s result, synthesis of a model library was initially performed by reacting a biguanide mixture (the fixed component) with a ketone (the varied component). The test library was synthesized by reacting a mixture of 6 arylbiguanides (phenyl, 4-methylphenyl, 4-chlorophenyl, 4-bromophenyl, 4-ethylphenyl and 3,4-dichlorophenylbiguanide) hydrochloride and excess of 4-heptanone in the presence of triethyl orthoacetate and conc.HCl. If the reaction was successful, 6 products would be formed in equal quantities with minimal side-reactions.

The use of TLC in the monitoring of the progression of the synthesis was not efficient enough to determine the number of products formed in each library. For this reason a HPLC analytical method was devised to ascertain the number of products in the library. The mixture of products collected from the model library was analyzed on a reverse-phase C_{18} column and eluted with a mobile phase containing a mixture of triethylammonium acetate buffer (0.1 M, pH 7) and acetonitrile as described previously (section **3.1**). This HPLC analytical technique was previously employed in analysis of the mixture of all 6 components in the model library (section **3.1**). Gratifyingly, the chromatogram revealed the presence of 6 products and only minor contaminants (arylbiguanide and rearrangement products) were observed. The result from HPLC analysis of the model sub-library **1** was as shown in **Figure 3.13**. The products were identified from the sequence of elution, depended on hydrophobic interaction of substituent group on the phenyl ring according to ref. 32. It is clearly seen that each peak possessed comparable peak area. Ignoring the minor the difference in molar absorptivity of each compound, it may be concluded that the method gave all six expected products in equimolar quantity. The presence of all six products was also confirmed mass spectral analysis which showed the expected 6 peaks (**Figure 3.14**).

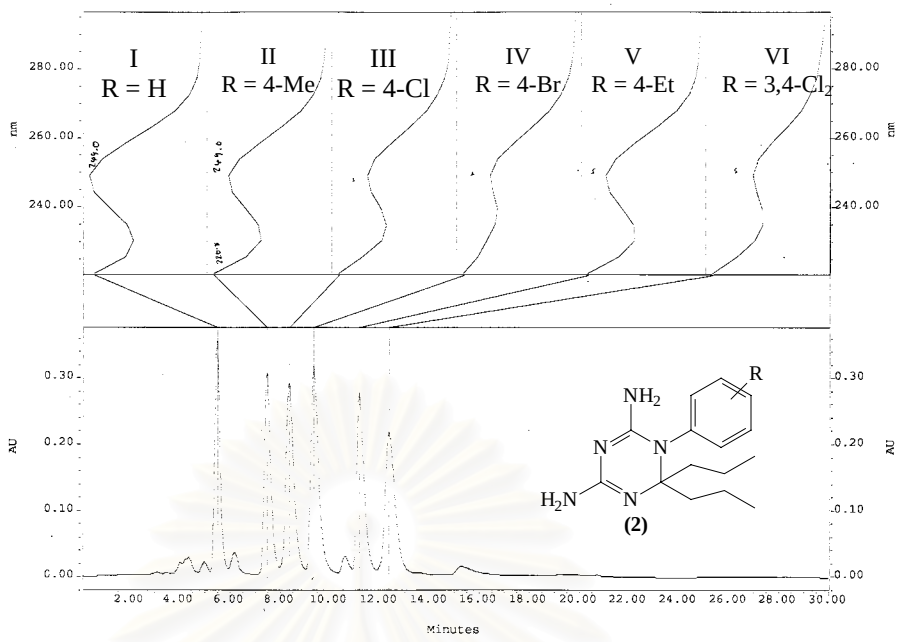


Figure 3.13: Spectrum and chromatogram from HPLC analysis of reaction between mixture of arylbiguanide and 4-heptanone

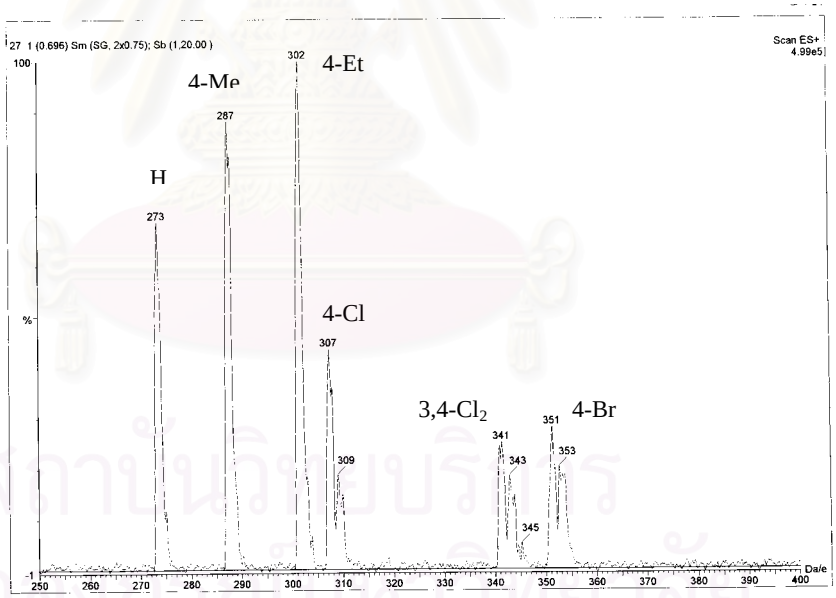
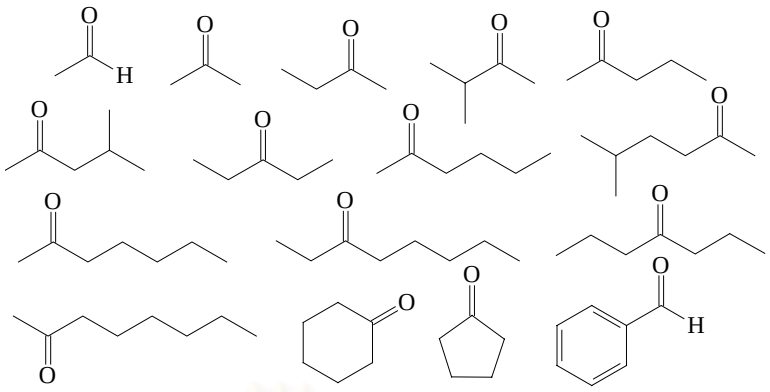
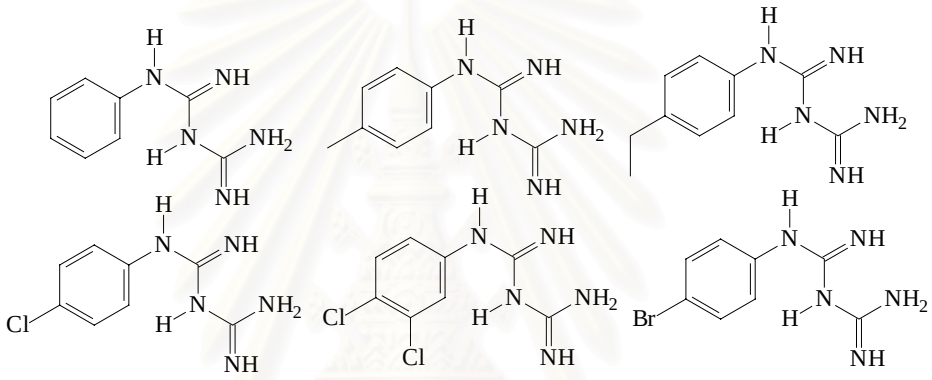


Figure 3.14: Mass spectral analysis of reaction between arylbiguanide and 4-heptanone

Having established the method for synthesizing the 4-heptanone sub-library, a 96-membered combinatorial library was designed and created from 6 biguanide and 16 carbonyl compounds (**Scheme 3.7**) employing the split synthesis strategy.



carbonyl compounds



arylbiguanides

Scheme 3.7: Structure of 6 biguanides and all carbonyl compounds

All 16 sub-libraries were synthesized using the optimized condition previously described (section **3.1**) except sub-library **16** (acetaldehyde as carbonyl compound). Because acetaldehyde was changed to the less reactive acetal by reaction with methanol in the presence of acid and TEOA, the desired dihydrotriazine products were not formed under the usual condition. However, if isopropanol was used and TEOA was omitted in synthesis of this sub-library, the reaction proceeded smoothly to give the expected 6-membered 2-methyl-dihydrotriazine sub-library. The reactions of were carried out at room temperature and the progress of reaction was closely monitored. The reaction was stopped when either all the reactants were used up or when there was no further consumption of the reactants as judged by TLC or HPLC analysis and by ammoniacal copper sulphate reagent. It was observed that the rate of reaction varied over a wide window of reaction time, ranging from 20 to 125 hrs

(**Table 3.2**). Nevertheless, analysis of all sub-libraries by HPLC and MALDI-TOF MS revealed that all 96 compounds were present. In most cases only 6 major peaks were observed in each sub-library except for those involving sterically hindered ketones including sub-library **2**, **7** and **8** when significant amounts of side-reactions were observed. In all reaction of libraries, the crude products from evaporation of the solvent were triturated with ether and then the off-white solid precipitates were collected by filtration and washed again with ether and air dried.

Table 3.2: Reaction time, % yield and K_i value of all 16 sub-libraries

Sub-library	R^1	R^2	reaction time (hr)	% yield	K_i (wt) (nM)	Rel. to Cyc	K_i (mut.) (nM)	Rel. to Cyc	K_i (mut.)/ K_i (wt)
1	Pr^n	Pr^n	120	80	470.1 ± 31.7	313.4	39546.2 ± 10152.6	30.1	84.1
2	Me	Pr^i	125	73	36.4 ± 1.4	24.3	27657.9 ± 2096.2	21	759.8
3	Me	Hex^n	120	81	1.6 ± 0.2	1.1	315.9 ± 60.4	0.2	197.4
4	Me	Me	120	80	2.4 ± 0.0	1.6	1428.1 ± 286.3	1.1	595
5	Me	Pr^n	72	79	6.1 ± 0.6	4.1	3498.8 ± 601.4	2.7	573.6
6	Me	$-(CH_2)_2CH(CH_3)_2$	72	82	3.9 ± 0.3	2.6	1104.1 ± 0.3	0.8	283.1
7	$-(CH_2)_4-$		72	88	18.9 ± 1.8	12.6	667.6 ± 98.5	0.51	35.8
8	Me	Bu^i	24	94	5.0 ± 0.3	3.3	4301.2 ± 246.0	3.3	860.2
9	Et	Pen^n	72	90	42.9 ± 2.6	31.9	12232.9 ± 437.8	9.3	255.4
10	Me	Pen^n	72	82	4.9 ± 0.4	3.3	1527.9 ± 181.3	1.2	1998.3
11	H	Ph	120	100	6.4 ± 1.0	4.3	146.3 ± 44.5	0.11	22.9
12	Et	Et	113	94	11.4 ± 1.7	7.6	5664.9 ± 352.2	4.3	496.9
13	$-(CH_2)_5-$		123	98	414.2 ± 57.6	276.1	5593.5 ± 867.3	4.3	13.5
14	Me	Bu^n	113	93	5.5 ± 0.3	3.7	2015.8 ± 120.4	1.5	366.4
15	Me	Et	48	82	4.0 ± 0.5	2.7	1938.9 ± 224.6	1.5	484.7
16*	H	Me	20	80	2.5 ± 0.2	1.7	35.5 ± 7.3	0.03	14.2

*Isopropanol 5 mL was used instead absolute MeOH and no TEOA was used

Inhibition of Wild-Type and A16VS108T pf DHFRs

All the 16 sub-libraries of dihydrophenyltriazine mixture were screened separately for inhibitory activity against the both of wild-type and A16VS108T pf DHFRs using the iterative deconvolution technique. The data in **Table 3.2** showed the inhibition constant (K_i) value of the inhibitors which substituents at C-2 were varied in each sub-library, while the substituent group at N-1 position was mixture of phenyl, 4-methylphenyl, 4-chlorophenyl, 4-bromophenyl, 4-ethylphenyl and 3,4-dichlocro-phenyl groups.

The results showed that most sub-libraries except **1** and **13** showed fairly good K_i to wild-type DHFR (< 100 nM). Almost of all library with bulky substituent at C-2 position including the dimethyl group led to the poor binding to A16VS108T mutant enzyme (very high K_i value). One exception to this principle is the sub-library **3**, in which 2-octanone was used as the carbonyl component. K_i value of this library was ~ 5-fold more effective against the A16VS108T pfDHFR than, but it inhibited the wild-type enzyme ~1.1-fold less effectively than, cycloguanil (K_i value ~ 1314 nM (mutant) and ~ 1.5 nM (wt))[14]. However, when one substituent at C-2 position was H (sub-libraries **11** and **16**), it was found that they are significantly more effective against A16VS108T pfDHFR when compared to cycloguanil but they generally inhibited the wild-type enzyme less effectively than cycloguanil.

The accuracy of screening library was confirmed by comparing difference of inhibition constant (K_i) value between a combinatorially synthesized sub-library **11** (H and Ph at C-2 position) and a simulated sub-library **11** (each product was individually synthesized and purified before intimately mixing to form sub-library). The result of testing revealed that there was only a small difference in K_i between both libraries (**Table 3.3)**.

Table 3.3: The compared inhibition constant (K_i) value between a combinatorially synthesized sub-library **11** and a simulate synthesized sub-library **11** (each product was synthesized and purified before was mixed to be sub-library)

Synthesized library	K_i (wt) (nM)	Rel. to Cyc	K_i (mut.) (nM)	Rel. to Cyc	K_i (mut.)/ K_i (wt)
Combinatorially synthesized sub-library **11**	6.4 ± 1.0	4.3	146.3 ± 44.5	0.11	22.9
Simulated sub-library **11**	6.0 ± 0.5	4	89.1 ± 15.7	0.07	14.8

The three most effective sub-libraries against both wild-type and A16VS108T mutant included sub-library **16** (acetaldehyde: K_i wt 2.5 ±0.2; K_i mut 35.5 ± 7.3 nm), **11** (benzaldehyde: K_i wt 6.4 ± 1.0; K_i mut 146.3 ± 44.3 nm) and **3** (2-octanone: K_i wt 1.6 ± 0.2; K_i mut 315.9± 60.4 nm) (**Figure 88)**. Therefore all 18 individual members of these sub-libraries were resynthesized. Only all members of sub-library **3** needed to be synthesized because all members of sub-library **11** and **16** were previously synthesized and tested activity against pfDHFRs (wild-type and A16VS108T mutant) [35]. The data in **Figure 3.15, 3.16** and **Table 3.4** showed that the 3,4-dichlorophenyl substituents at N-1 position of all three sub-libraries were more effective against A16VS108T mutant pfDHFR than the other substituents. From these three sub-libraries, two compounds which are quite effective against both wild-type and mutant DHFR have been identified. These included **(2a)** and **(2b)** (**Figure 3.17)**. Both compounds are comparably effective to wild-type DHFR to cycloguanil but are about 100-times more active against A16VS108T mutant. It is interesting to note that both compounds carry 3,4-dichlorophenyl substituent at N-1 and one hydrogen substituent at C-2, the fact that is consistent with previous observation.[35]

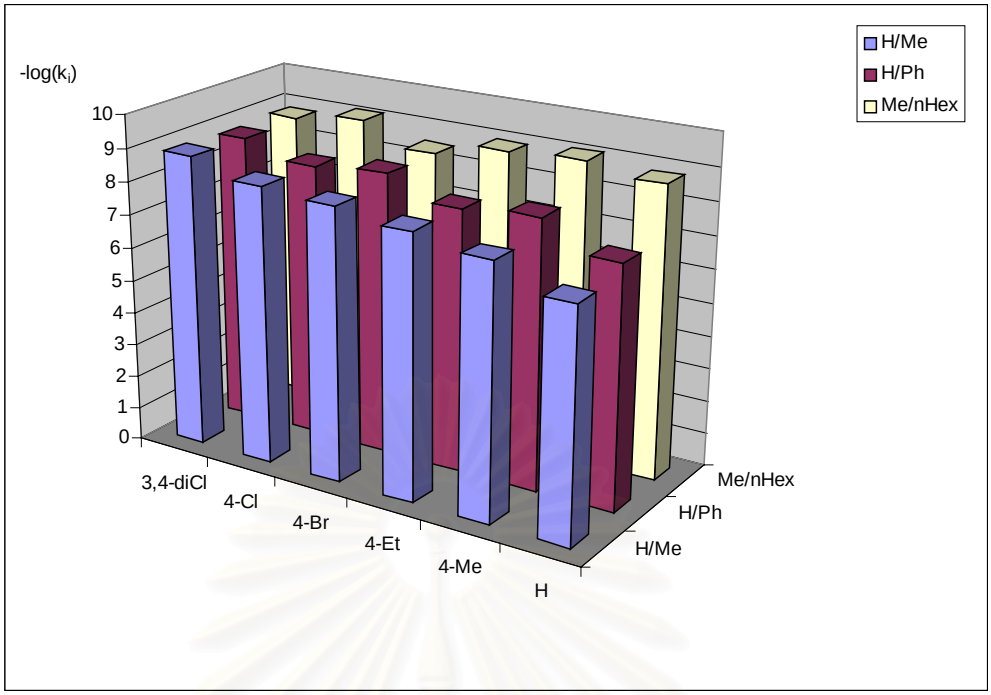


Figure 3.15: 3-D plot showing relation of –log(K_i) of each member of the three most effective sub-libraries against wild-type DHFR from *P.falciparum*

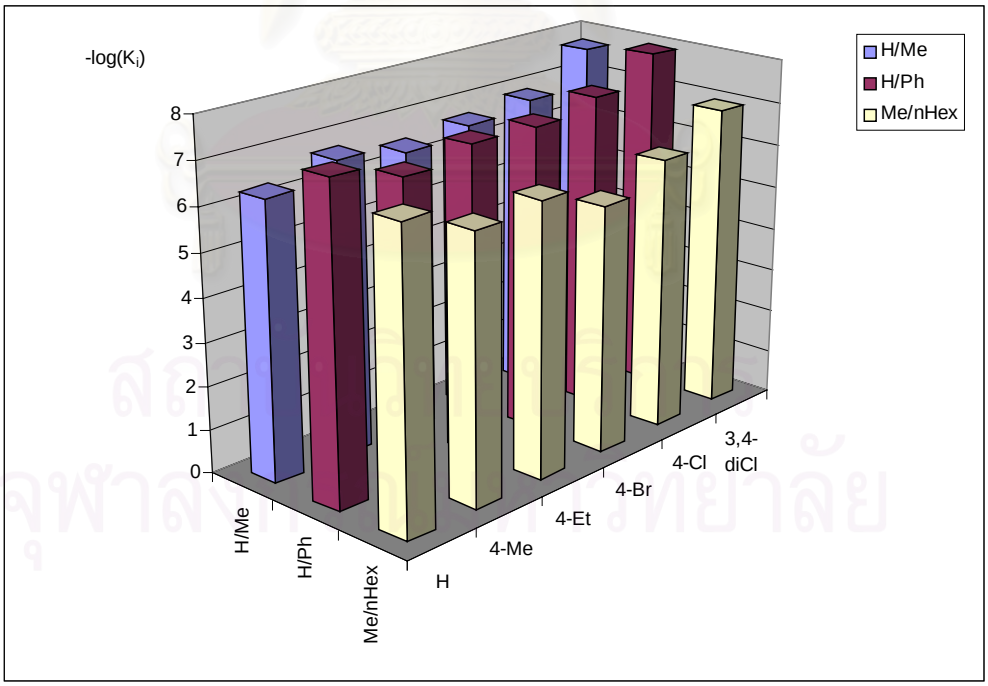
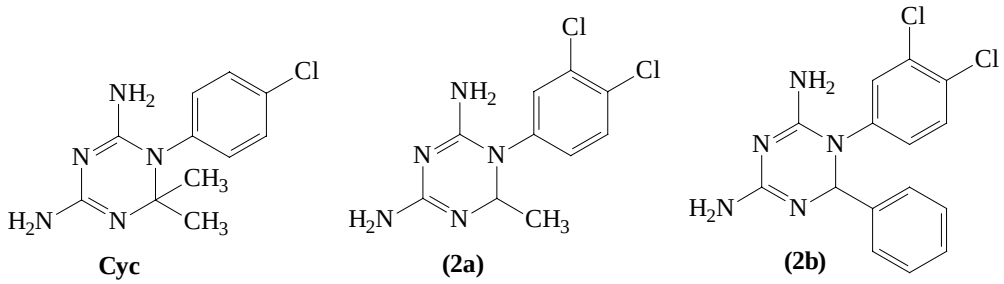


Figure 3.16: 3-D plot showing relation of –log(K_i) of each member of the three most effective sub-libraries against A16VS108T mutant DHFR from *P.falciparum*

Table 3.4: K_i of each member of the three most effective sub-libraries against wild-type and A16VS108T mutant DHFRs from *P.falciparum* (see **Table 3.2**).

Sub-library	**R^1**	**R^2**	**Ar** at N-1	K_i (wt)[a] (nM)	K_i (mut.)[b] (nM)
16	H	Me	Ph	113.1 ± 14.8[c]	456.3 ± 49.0[c]
			4-MeC_6H_5	23.4 ± 1.9[c]	185.5 ± 22.4[c]
			4-ClC_6H_5	4.1 ± 0.0[c]	126.7 ± 13.6[c]
			4-BrC_6H_5	5.7 ± 0.5[c]	201.7 ± 16.9[c]
			4-EtC_6H_5	11.0 ± 3.0[c]	322.1 ± 56.4[c]
			3,4-$Cl_2C_6H_3$	1.4 ± 0.2[c]	17.8 ± 0.8[c]
11	H	Ph	Ph	49.1 ± 3.5[c]	61.8 ± 15.6[c]
			4-MeC_6H_5	7.7 ± 2.2[c]	170.4 ± 13.8[c]
			4-ClC_6H_5	4.5 ± 0.2[c]	49.3 ± 3.3[c]
			4-BrC_6H_5	2.9 ± 1.2[c]	90.3 ± 11.4[c]
			4-EtC_6H_5	12.1 ± 2.3[c]	86.6 ± 10.8[c]
			3,4-$Cl_2C_6H_3$	1.6 ± 0.2[c]	11.0 ± 1.8[c]
3	Me	-Hex^n	Ph	1.5 ± 0.8	180.2 ± 12.6
			4-MeC_6H_5	0.8 ± 0.6	823.1 ± 154.8
			4-ClC_6H_5	0.7 ± 0.5	563.6 ± 150.7
			4-BrC_6H_5	3.0 ± 1.8	2186.3 ± 338.2
			4-EtC_6H_5	1.1 ± 0.4	551.9 ± 39.4
			3,4-$Cl_2C_6H_3$	1.4 ± 0.2	106.8 ± 32.2

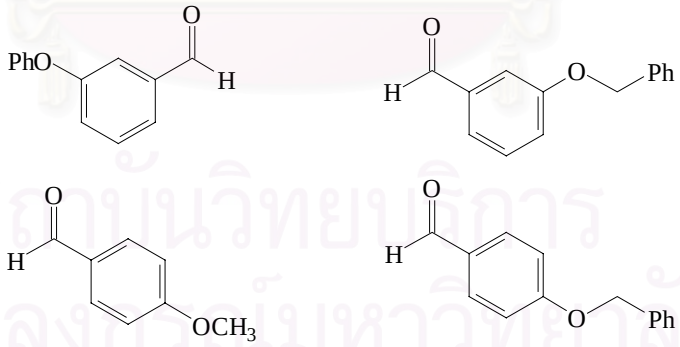
[a] Wild-type, [b] A16VS108T mutant pfDHFR, [c] Data from ref. 35



K_i (wt) = 1.5 ± 0.3 nM	K_i (wt) = 1.4 ± 0.2 nM	K_i (wt) = 1.6 ± 0.2 nM
K_i (mut) = 1,314 ± 16	K_i (mut) = 17.8 ± 0.8	K_i (mut) = 11.0 ± 1.8

Figure 3.17: Structure of **(2a)** and **(2b)** which have highest activity against wild-type and A16VS108T mutant pfDHFR from the 16 sub-libraries

The sub-library 11 bearing the C-2 phenyl substituent deriving from benzaldehyde appeared to give very good activity. We envisaged that further diversity could be generated by using substituted benzaldehyde as starting meterials in order to find an even better inhibitors. Consequently, a second set of library was created from 4 substituted aromatic aldehydes (**Scheme 3.8**) and the 6 biguanides previously employed to create the first set of library. The synthesis data was shown in **Table 3.5**



Scheme 3.8: Structure of 4 substituted aromatic aldehydes

Table 3.5: Reaction time, % yield and K_i value of **17-20** sub-libraries

Sub-library	R^1	R^2	reaction time (hr)	% yield	K_i (wt) (nM)	Rel. to Cyc	K_i (mut.) (nM)	Rel. to Cyc	K_i (mut.)/ K_i (wt)
17	H	3-$PhOC_6H_4$	>10 days	81	0.3 ± 0.0	0.2	1.6 ± 0.2	0.001	5.3
18	H	4-$PhCH_2O$ C_6H_4	48	83	1.7 ± 0.3	1.1	7.05 ± 0.9	0.005	4.1
19	H	4-CH_3O C_6H_4	72	88	14.0 ± 1.8	9.3	166.6 ± 51.0	0.13	11.9
20	H	3-$PhCH_2O$ C_6H_4	120	57	2.2 ± 0.8	1.47	12.8 ± 4.8	0.01	5.8

Screening the 4 sub-libraries with wild-type and A16VS108T mutants pfDHFRs revealed that they are very effective inhibitors. The two best sub-libraries from the first-round screening included 3-phenoxybenzaldehyde (**17**: K_i wt 0.3 ± 0.0; K_i mut 1.6 ± 0.2 nm) and 4-benzyloxybenzaldehyde (**18**: K_i wt 1.7 ± 0.3; K_i mut 7.1 ± 0.9 nm) respectively (**Table 3.5**). All members of each sub-library were synthesized again as 12 individual compounds and the K_i values against wild-type and mutant enzymes were determined.

Table 3.6: K_i of each member of the first two most effective sub-libraries against wild-type and A16VS108T mutant DHFRs from *P.falciparum* (see **Table 3.2**).

Sub-library	R^1	R^2	**Ar** at N-1	K_i (wt)[a] (nM)	K_i (mut.)[b] (nM)
17	H	3-PhOPh	Ph	5.4 ± 2.1	2.2 ± 1.2
			4-MeC_6H_5	2.7 ± 0.3	2.4 ± 0.5
			4-ClC_6H_5	0.6 ± 0.1	3.5 ± 0.4
			4-BrC_6H_5	3.0 ± 1.2	4.0 ± 0.5
			4-EtC_6H_5	4.9 ± 1.7	2.0 ± 1.5
			3,4-$Cl_2C_6H_3$	1.9 ± 0.3	5.2 ± 0.6
18	H	4-$PhCH_2OPh$	Ph	5.41 ± 1.3	8.0 ± 0.8
			4-MeC_6H_5	1.02 ± 0.2	11.1 ± 3.9
			4-ClC_6H_5	1.25 ± 0.3	10.9 ± 1.0
			4-BrC_6H_5	1.38 ± 0.2	12.2 ± 1.9
			4-EtC_6H_5	1.4 ± 0.2	8.86 ± 1.8
			3,4-$Cl_2C_6H_3$	2.16 ± 0.7	10.7 ± 1.4

[a] Wild-type, [b] A16VS108T mutant pfDHFR

The data in **Table 3.6** revealed that all members in sub-libraries were comparably effective against both enzymes, especially for sub-library **17**. It may be because the effect of substituent group at C-2 position had dominated the effect of substituent group at N-1 position. Therefore no good inhibitors could be selected indicating the limitation of combinatorial method in identifying the best inhibitor from a pool of similarly active compounds. Nevertheless, almost all compounds exhibited better activities compared to **(2b)** which are the lead inhibitor and over 100-times more effective than cycloguanil against the A16VS108T pfDHFR.

3.6 Synthesis of combinatorial libraries of 4,6-diamino-1,2-dihydro-1,3,5-triazine (used mixture of 11 ketone compounds)

There were also attempts to explore the possibilities of creating combinatorial libraries in a complementary fashion, *ie*, by reacting a biguanide with a mixture of carbonyl compounds. However, the developed synthetic methodology required that the carbonyl compound must be used in excess in order to drive the reaction to completion. The complicating factor is that each carbonyl compound has different reactivities. Hence, the isokinetic mixture of carbonyl compound must be prepared in the first step. Eleven carbonyl compounds were divided in to three groups according to their relative reactivities. Each group containg equimolar mixture of carbonyl compounds was reacted with 4-chlorophenylbiguanide in the presence of triethyl orthoacetate and HCl in methanol as described previously. Two ketones, ie, 3-octanone and 2-heptanone were included in all sets to be used as markers. Distributions of the products were analyzed by HPLC and the ratio of ketones was adjusted until an equimolar mixture of the product was obtained. The best ratio of mixture of 2-pentanone:2-hexanone:2-heptanone:5-methyl-2-hexanone:2-octanone:3-octanone in the first group was 1.0:1.0:1.0:1.0:1.0:3.6 eq. In the second group, the best ratio of mixture of 3-methyl-2-butanone:2-heptanone:3-octanone was 3.8:1.0:3.6 eq. In the third group, the best ratio of mixture of acetone:2-butanone:3-pentanone:4-methyl-2-pentanone:2-heptanone:3-octanone was 2.9:3.0:8.1:5.6:2.5:9.0 eq. After that the isokinetic ratio of all 11 ketones was obtained by normalizing the ratio using the markers to give the final ratio of 2-pentanone:2-hexanone:2-heptanone:5-methyl-2-hexanone:2-octanone:3-octanone:3-pentanone:4-methyl-2-pentanone:2-butanone:acetone:3-methyl-2-butanone 2.5:2.5:2.5:2.5:2.5:9:8.1:5.6:3.0:2.9:9.4 mmol.

This ratio was used as an isokinetic mixture to react with a biguanide. Nevertheless, the 11 products were not formed in equal proportion (**Figure 89**). Therefore, the combinatorial library could not be successfully synthesized by this method.

CHAPTER IV

CONCLUSION

An efficient method for the synthesis of solution phase combinatorial mixture of 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazine was developed. The effect of three parameters including ratio of reactants, amounts of acid catalyst and temperature have been studied in a model reaction between 4-chlorophenylbiguanide hydrochloride and methyl isobutyl ketone in the presence of triethyl orthoacetate as water scavenger. The optimized reaction condition at 1 mmol of 4-chlorophenylbiguanide hydrochloride was found to be: 5 mmol of methyl isobutyl ketone, 0.75 mL of triethyl orthoacetate and 0.023 mL of conc.HCl at room temperature (30 $^{\circ}$C). The conditions were tested with several other systems, and in all case the desired product were obtained in fair to good yield. This optimized condition was used in the synthesis of combinatorial mixture of 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazine. By split synthesis strategy, reaction between 6 arylbiguanides hydrochloride and 16 carbonyl compounds gave a 96-membered combinatorial library as 16-sub-libraries containing 6 compounds each. Analysis of the sub-libraries by HPLC and mass spectrometry confirmed that all compounds were present more-or-less equally. Screening by iterative deconvolution method indicated that 1-(3′,4′-dichlorophenyl)-2-methyl and 1-(3′,4′-dichlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-traizine possesed highest activity against wild-type and A16VS108T mutant *Plasmodium falciparum* dihydrofolate reductase enzymes. Further 24-membered library was also created from the same 6 arylbibuanides and 4 aromatic aldehydes. Screening of the library revealed that all 2-(3′-phenoxyphenyl) and 2-(4′- phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-traizine products possesed even higher activity than the two lead inhibitors obtained earlier. Unfortunately, the best inhibitors could not be identified that was because effect of 3′-phenoxyphenyl at C-2 position dominate the substituent group on phenyl ring at N-1 position. All best inhibitors identified are comparably effective to cycloguanil against wild-type pfDHFR but around 70-650 folds more effective than cycloguanil against A16VS108T mutant pfDHFR.

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APPENDICES

Table 1: Peak areas of **(1)-(4)** according to HPLC analysis; when 0.19 mL (1.5 eq.) of MIBK was used

Time (hr)	Area (uV*sec)			
	(1)	**(2)**	**(3)**	**(4)**
0.5	11005918	924292	55380	0
1.0	13170629	1964443	184547	0
2.0	17278961	4376044	561806	0
3.0	11036765	4487432	334226	82770
4.0	12216911	5937620	460545	118514
7.0	12365657	8729659	820780	198470
10.0	6102495	5116622	580044	182422
13.0	12108527	10616474	1480134	476256
18.0	19605430	15080630	3136659	1016920
23.0	9406549	7414505	1675902	568223
28.0	10121927	8008555	2172262	728378
33.0	7104222	5634312	1746912	591794
38.0	6761011	5420558	1884829	626662
43.0	7749948	6166552	2392389	802915
48.0	9340992	7441046	3200067	1078918
51.5	9053136	6729668	3007942	985699

Table 2: Peak areas of **(1)-(4)** from HPLC analysis; when 0.31 mL (2.5 eq.) of MIBK was used

Time	Area (uV*sec)			
(hr)	(1)	(2)	(3)	(4)
1.0	9887219	1264962	112263	0
3.0	8792711	2905411	193356	55866
6.0	6840287	4389671	456954	119448
10.0	5792539	5002254	759375	172401
12.0	5246603	5147676	781856	183426
29.0	3955356	4228788	1187684	281226
48.5	4613272	4688910	2390255	515111
52.5	4338692	4501088	2426646	538478

Table 3: Peak areas of **(1)-(4)** from HPLC analysis; when 0.63 mL (5 eq.) of MIBK was used

Time	Area (uV*sec)			
(hr)	(1)	(2)	(3)	(4)
1.0	7940371	2200368	117396	0
3.0	5190350	6240559	536379	61667
6.0	2490124	6724560	668417	77372
10.0	1662356	6120909	802125	89565
12.0	2012472	8141631	1207164	119185
29.0	2046136	6346291	1882076	189811
48.5	1940053	4882549	2427552	237198
52.5	2362367	6250496	3359657	328021

Table 4: Peak areas of **(1)-(4)** from HPLC analysis; when 1.24 mL (10 eq.) of MIBK was used

Time	Area (uV*sec)			
(hr)	(1)	(2)	(3)	(4)
1.0	9947777	2645283	300582	0
3.0	3301660	5095113	482421	31943
6.0	1724504	6827935	787985	63575
10.0	1588983	8337438	1297949	79595
12.0	1946475	10190402	1679254	125414
29.0	1313281	6529194	2068096	115252
42.5	2164038	7794386	4246244	231327
52.5	2462428	9082907	5709402	304013

Table 5: Peak areas of **(1)-(4)** from HPLC analysis; when TEOA was not used in the reaction

Time	Area (uV*sec)			
(hr)	(1)	(2)	(3)	(4)
1.0	17353436	133699	0	0
4.0.	13552635	404127	0	0
10.0	14176000	1051989	175661	0
22.5	12592095	2070907	422056	0
30.0	14956146	3194176	809645	0
48.0	10046278	3294176	102920	0
63.0	9070266	3678368	1314496	0

Table 6: Peak areas of **(1)-(4)** from HPLC analysis; when TEOA was 0.35 mL/mmol

Time	**Area (uV*sec)**			
(hr)	**(1)**	**(2)**	**(3)**	**(4)**
1.0	15303419	833647	0	0
4.0	13202307	255292	289278	137599
10.0	8423541	3587929	444917	200893
12.0	10921399	5122021	674327	311995
24.5	7575430	6067712	1079062	431322
29.5	7383528	6413025	1496712	534737
52.5	7259923	8156623	2872844	928928

Table 7: Peak areas of **(1)-(4)** from HPLC analysis; when TEOA was 0.7 5 mL/mmol

Time	**Area (uV*sec)**			
(hr)	**(1)**	**(2)**	**(3)**	**(4)**
1.0	8354622	6651562	589610	0
4.0	1901197	8859428	881689	67500
10.0	1938999	8874153	1228318	59424
12.0	1890785	8990979	1355192	82979
24.5	2101661	9173634	2132056	154950
29.5	2596775	8197215	2368130	131595
52.5	1844580	8389311	3628722	234416

Table 8: Peak areas of **(1)-(4)** from HPLC analysis; when TEOA was 1 mL/mmol

Time	Area (uV*sec)			
(hr)	(1)	(2)	(3)	(4)
1.0	3085488	7016204	603620	47744
4.0	1556485	10986349	1239898	135265
10.0	1288116	9442630	1415085	51045
12.0	1486165	9962813	1646072	138133
24.5	826870	8425989	1925629	115736
29.5	859759	8924235	2529844	123581
52.5	1473264	17395933	7779854	340602

Table 9: Peak areas of **(1)-(4)** from HPLC analysis; when HCl was 0.023 mL

Time	Area (uV*sec)			
(hr)	(1)	(2)	(3)	(4)
1.0	11452681	3577003	107253	89990
4.0	1632491	7306602	1121820	68813
10.0	233838	7959051	1419814	97592
25.0	232252	8819992	2496912	122937
32.0	354939	10854438	3404153	172054
52.0	287958	8745283	3827940	130595

Table 10: Peak areas of **(1)-(4)** from HPLC analysis: at reaction temperature = 30°C

Time	Area (uV*sec)			
(hr)	(1)	(2)	(3)	(4)
1.0	18257938	1743575	161995	0
4.0	13242085	8945331	776319	0
10.0	3239087	9268468	639829	0
23.5	1747252	11075623	928804	0
29.5	1432897	8308037	738775	0
47.0	981723	6633404	715279	0

Table 11: Peak areas of **(1)-(4)** from HPLC analysis: at reaction temperature = 64 °C

Time	**Area (uV*sec)**			
(hr)	**(1)**	**(2)**	**(3)**	**(4)**
1.0	1726793	6069845	758559	0
2.5	3063374	12701061	2693339	0
5.5	2308325	6108464	2869890	0
8.2	4461111	6285483	5091264	157807

Table 12: Peak area of **(1)-(4)** from HPLC analysis under optimized condition

Time	**Area (uV*sec)**			
(hr)	**(1)**	**(2)**	**(3)**	**(4)**
1.0	18471856	1492833	115618	80315
4.0	7740605	3778073	275487	50869
10.0	1184617	6915813	570442	42955
24.0	330112	6515161	537798	22184
52.0	483832	8801970	1253904	18479

Table 13: Peak areas of all substances from HPLC analysis when acetone was used as the carbonyl component.

Time	**Area (uV*sec)**		
(hr)	**(1)**	**(2b)**	**(3b)**
1.0	11221393	487698	28625
4.0	12392437	2823184	169324
10.0	10230735	12883147	523056
24.3	226409	4750810	216889
33.3	114863	6039096	284144

Table 14: Peak areas of all substances from HPLC analysis when 3-methyl-2-butanone was used as the carbonyl component.

Time	Area (uV*sec)		
(hr)	(1)	(2c)	(3c)
1.0	16390446	198394	0
4.0	13370448	765215	93458
10.0	13540285	2184687	226681
24.0	6563747	3417319	308932
52.0	3564750	5302354	545200

Table 15: Peak areas of all substances from HPLC analysis when 4-heptanone was used as the carbonyl component.

Time	Area (uV*sec)		
(hr)	(1)	(2d)	(3d)
1.0	16551564	356971	0
10.0	12243967	2909731	291496
24.0	4743299	4380515	445480
52.0	1508801	10818533	879085
72.0	461778	9840254	873697

Table 16: Peak areas of all substances from HPLC analysis when 3-octanone was used as the carbonyl component.

Time	Area (uV*sec)		
(hr)	(1)	(2e)	(3e)
1.0	12717452	18123	0
10.0	10957990	1784305	176376
24.0	5753469	3634447	338007
52.0	2411279	9229317	91201
72.0	781757	10134978	957098

Table 17 Peak areas of all substances from HPLC analysis when benazldehyde was used as the carbonyl component

Time	**Area (uV*sec)**		
(hr)	**(1)**	**(2f)**	**(3f)**
3.0	20123527	142271	0
6.0	13313610	763434	0
10.0	14628848	2089207	173208
23.5	9472985	3764015	268154
32.5	9478222	7303163	517360
51.5	2998908	7205519	466213
76.5	0	8137295	278775

Table 18: Peak areas of all substances from HPLC analysis when biguanide **(1h)** was used in the reaction

Time	**Area (uV*sec)**		
(hr)	**(1h)**	**(2h)**	**(3h)**
1.0	3142455	350427	16917
4.0	4545151	1989290	150609
10.0	3357212	4398505	0
24.0	1236144	2879954	199976
52.0	982333	4883953	267867

Table 19: Peak areas of all substances from HPLC analysis when biguanide **(1i)** was used in the reaction

Time	**Area (uV*sec)**		
(hr)	**(1i)**	**(2i)**	**(3i)**
1.0	10476216	454263	24246
4.0	10117384	2185001	258437
10.0	3575211	4233718	374098
24.0	354140	4242946	330904
52.0	173857	7510622	659850

Table 20: Peak areas of all substances from HPLC analysis when biguanide **(1j)** was used in the reaction

Time	**Area (uV*sec)**		
(hr)	**(1j)**	**(2j)**	**(3j)**
1.0	13870519	1606952	99577
4.0	5698956	5467986	394180
10.0	1122440	7059348	569655
24.0	330270	4541243	363813
52.0	622102	6976892	1187201

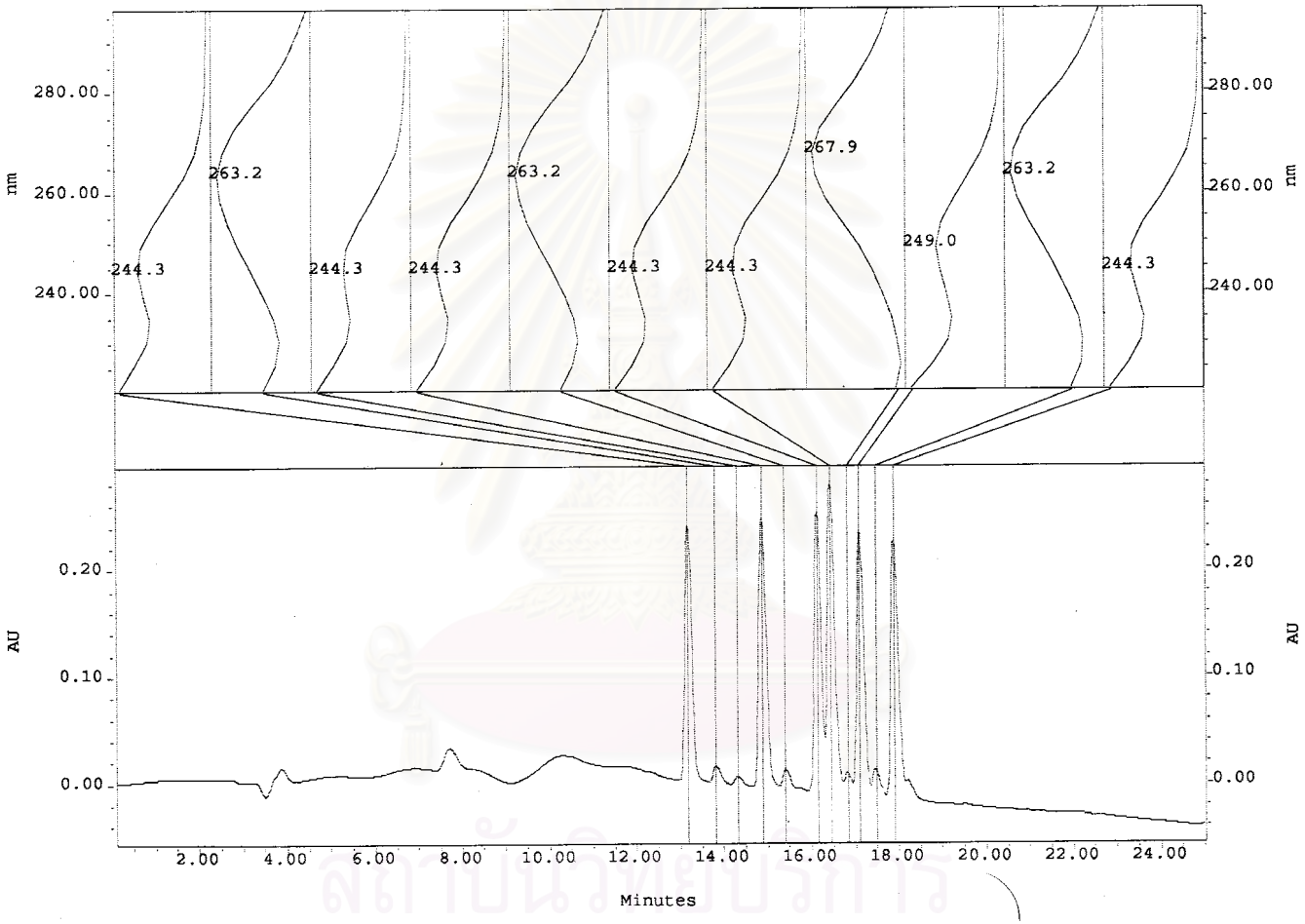

Figure 1: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of 2-pentanone: 2-hexanone:2-heptanone:5-methyl-2-hexanone:2-octanone:3-octanone in 1.0:1.0:1.0:1.0:1.0:3.6 ratio **(1-b)** and 4-chlorophenylbiguanide hydrochloride

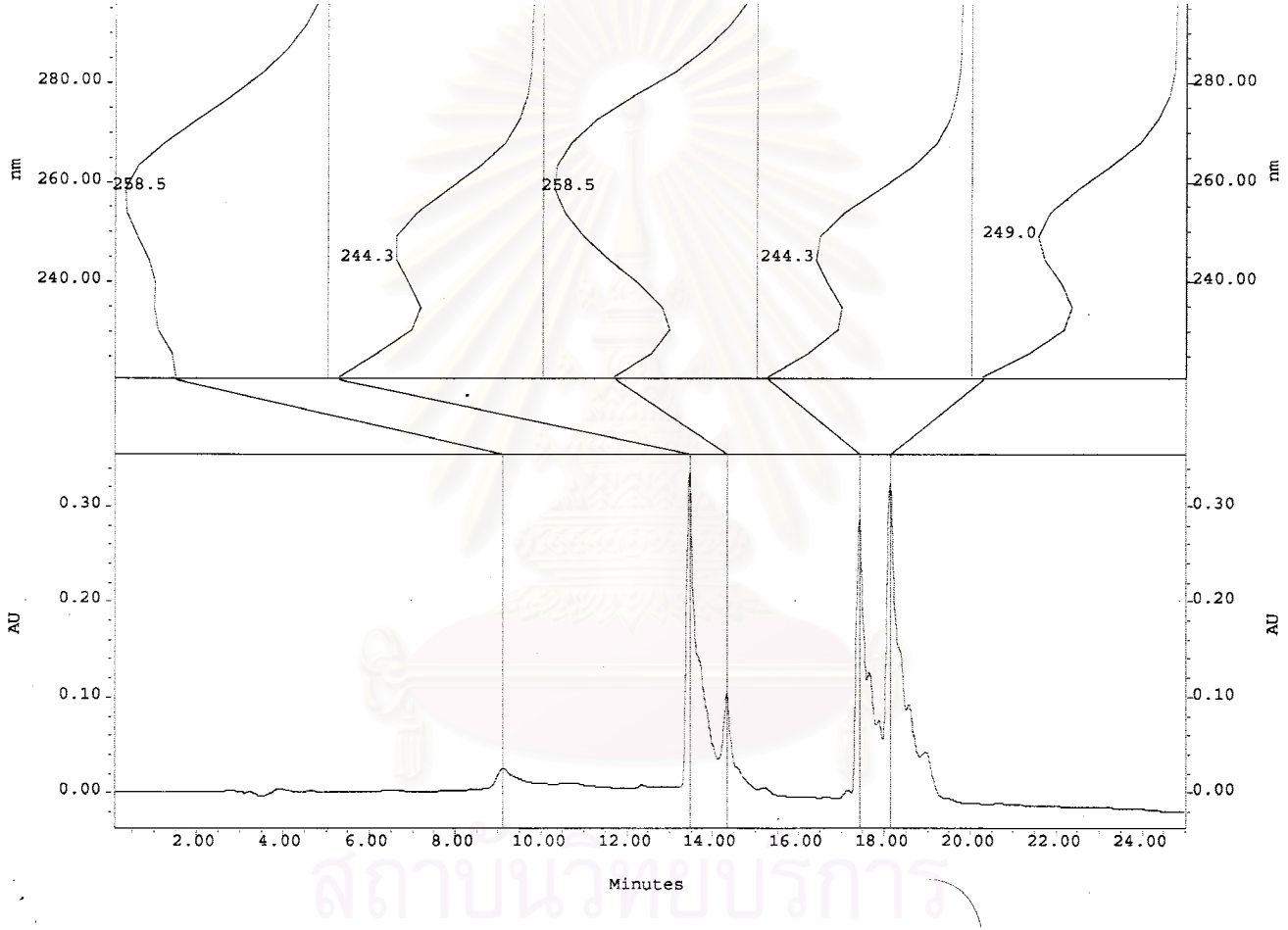

Figure 2: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of 3-methyl-2-butanone:2-heptanone:3-octanone in 3.8:1.0:3.6 ratio **(2-c)** and 4-chlorophenylbiguanide hydrochloride

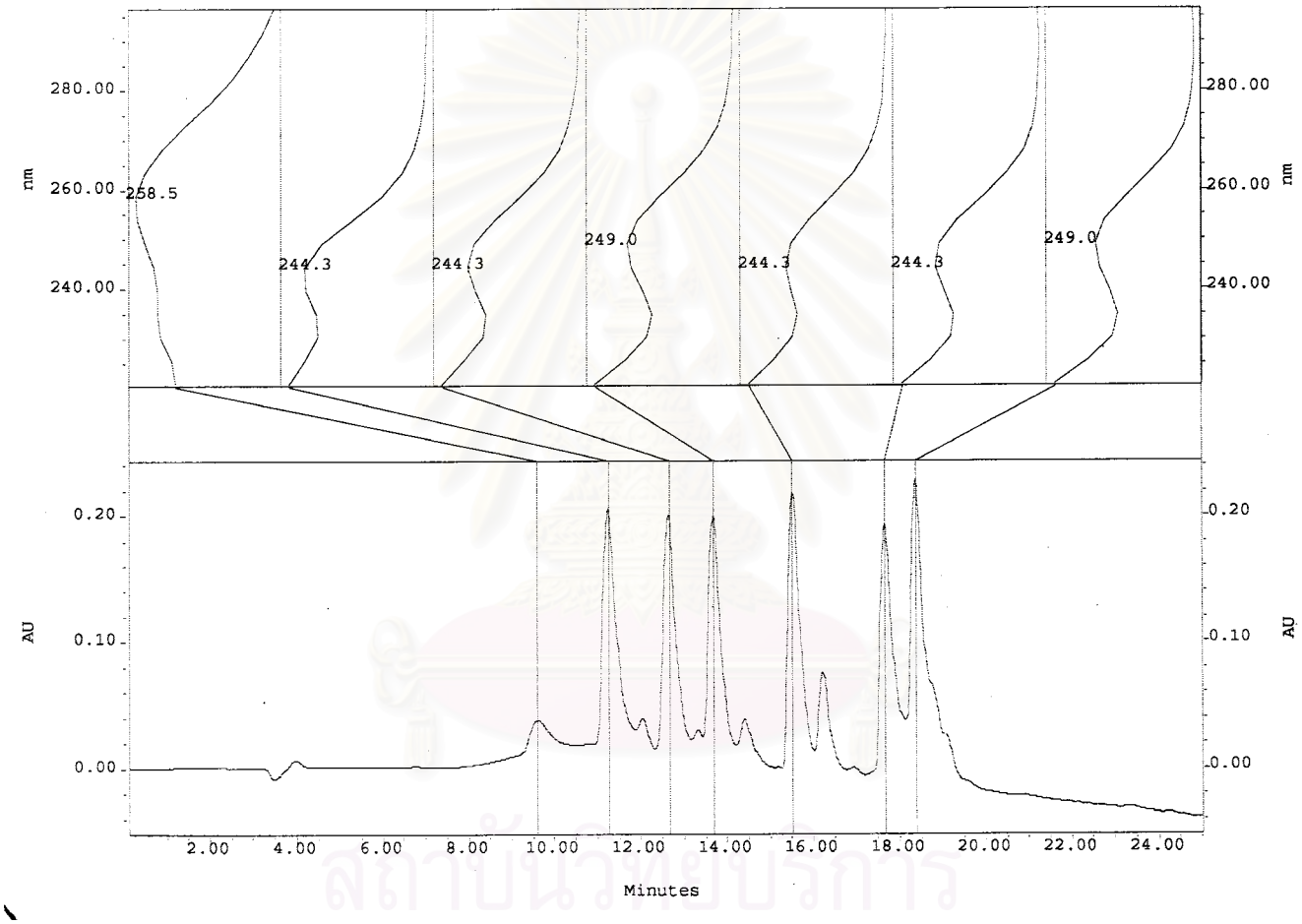

Figure 3: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of acetone:2-butanone:3-pentanone:2-heptanone:3-octanone in 2.9:3.0:8.1:5.6:2.5:9.0 ratio **(3-g)** and 4-chlorophenylbiguanide hydrochloride

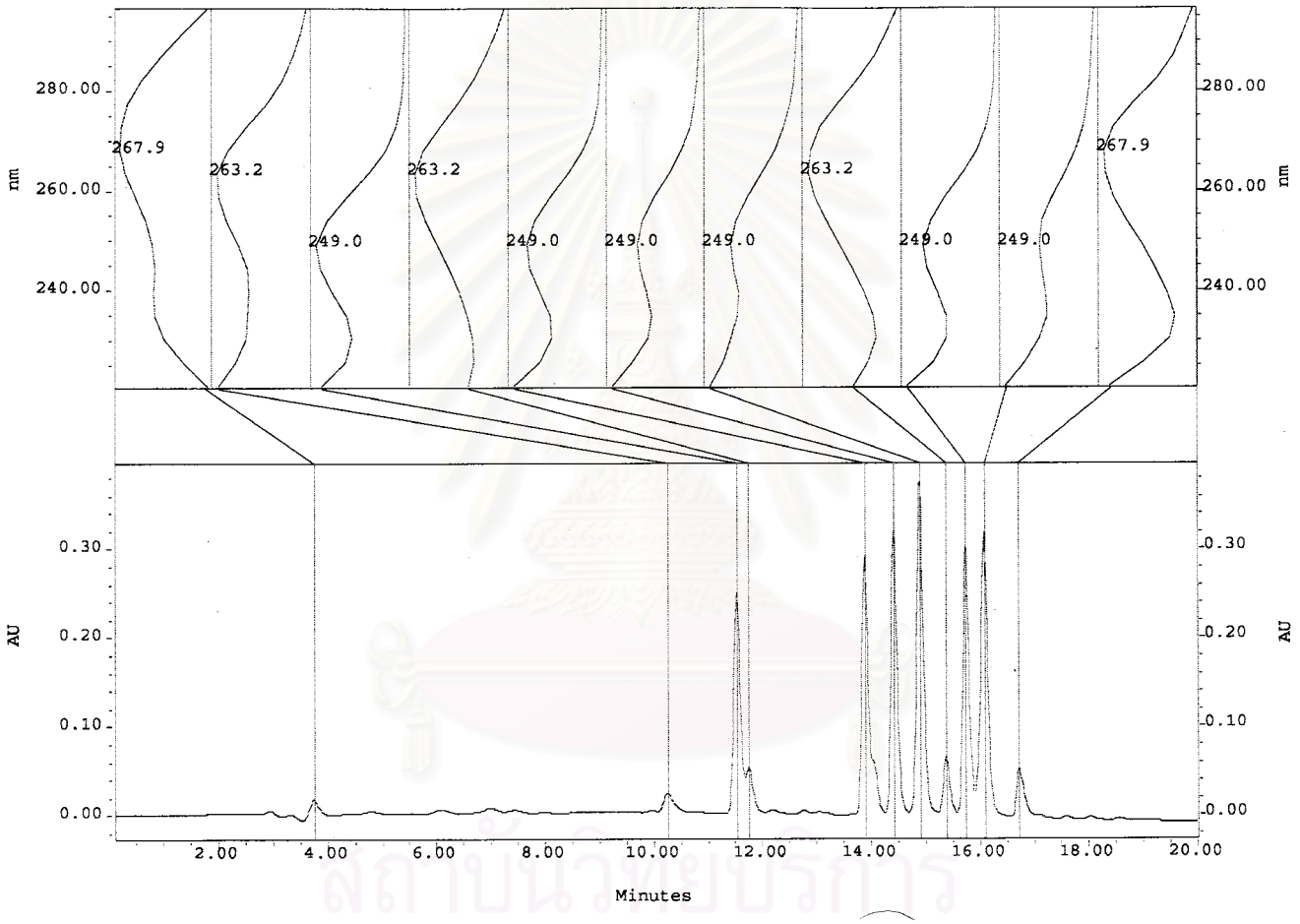

Figure 4: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and 4-heptanone (sub-libraries **1**)

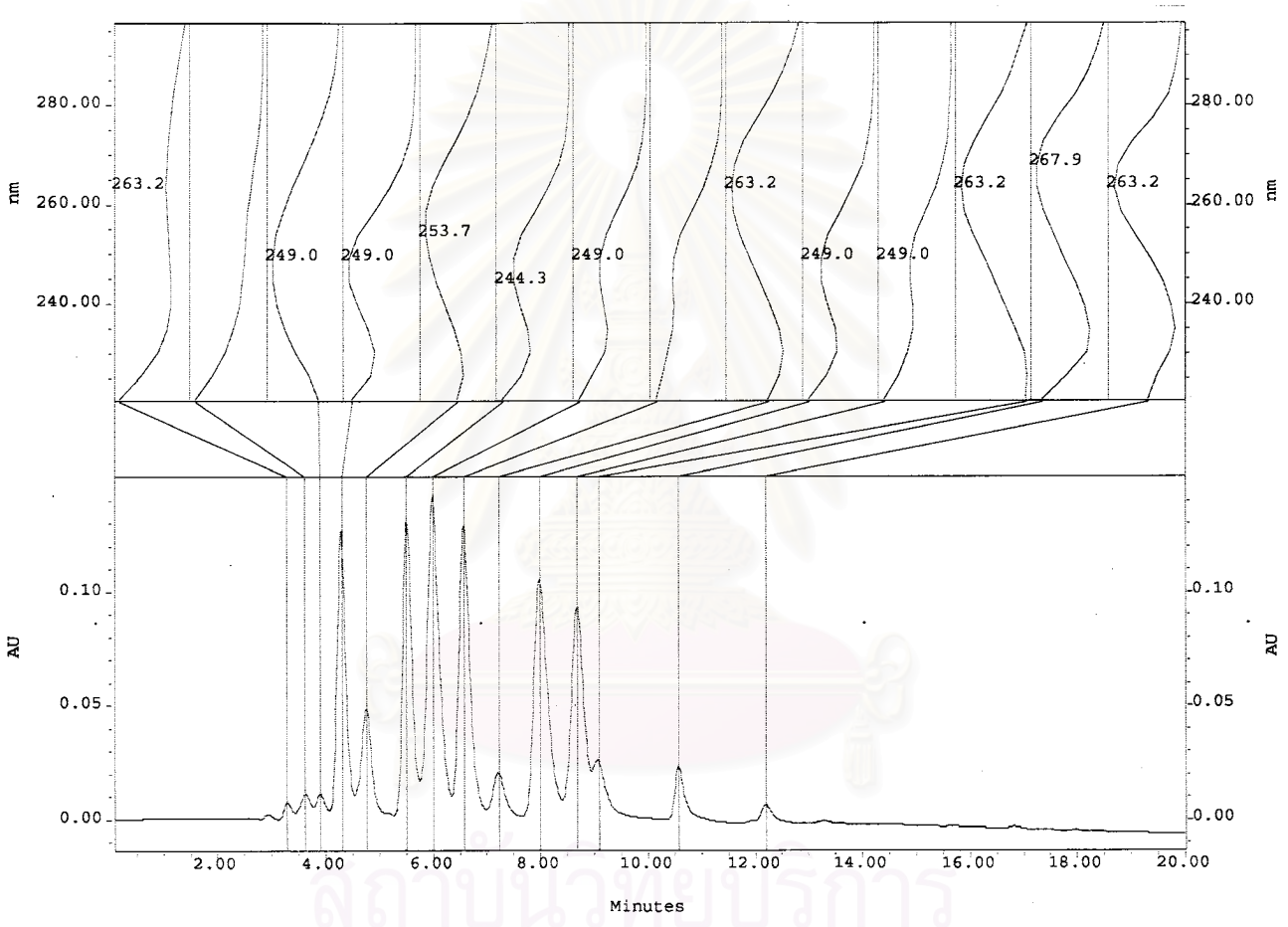

Figure 5: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and 3-methyl-2-butanone (sub-libraries **2**)

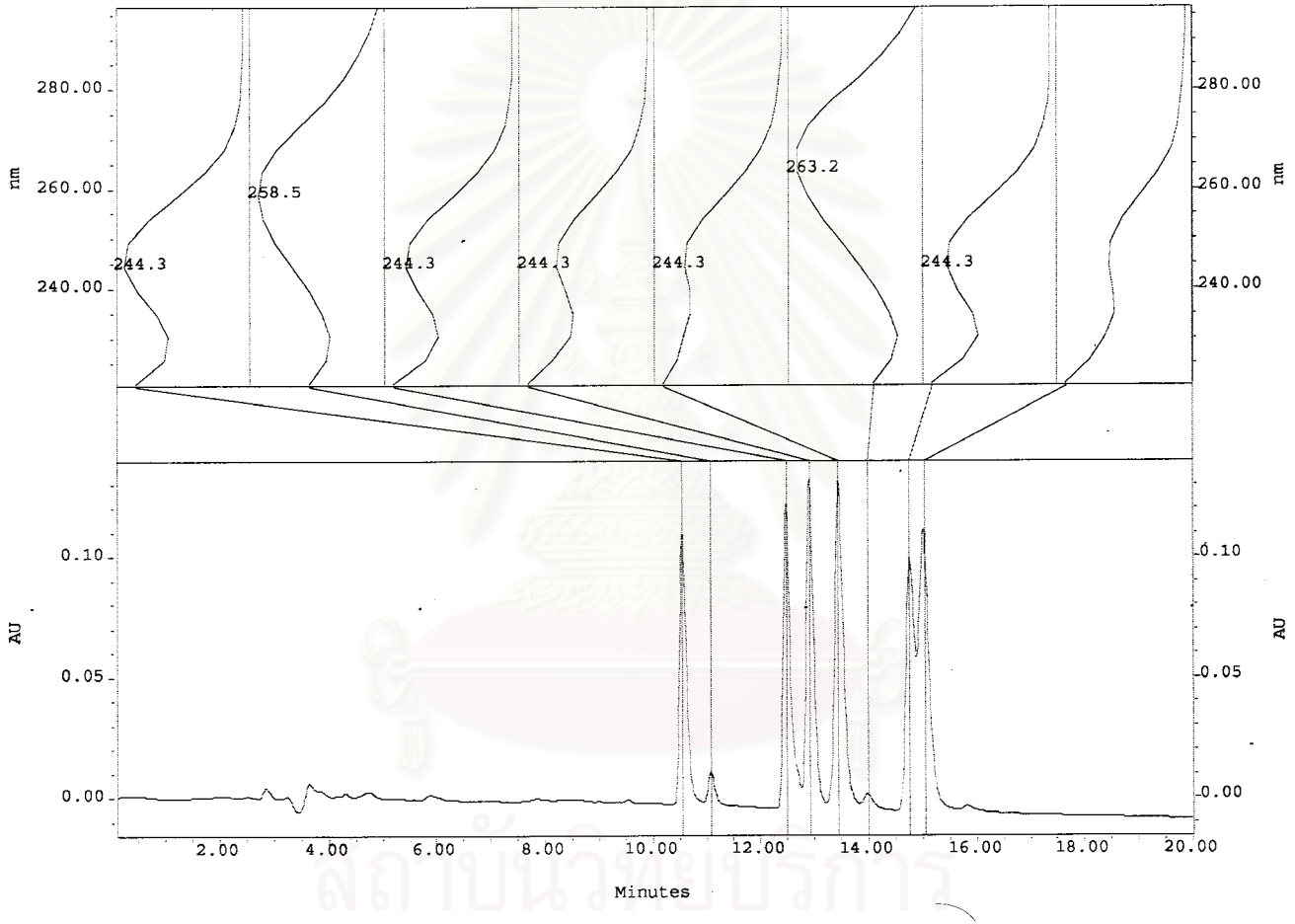

Figure 6: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and 2-octanone (sub-libraries **3**)

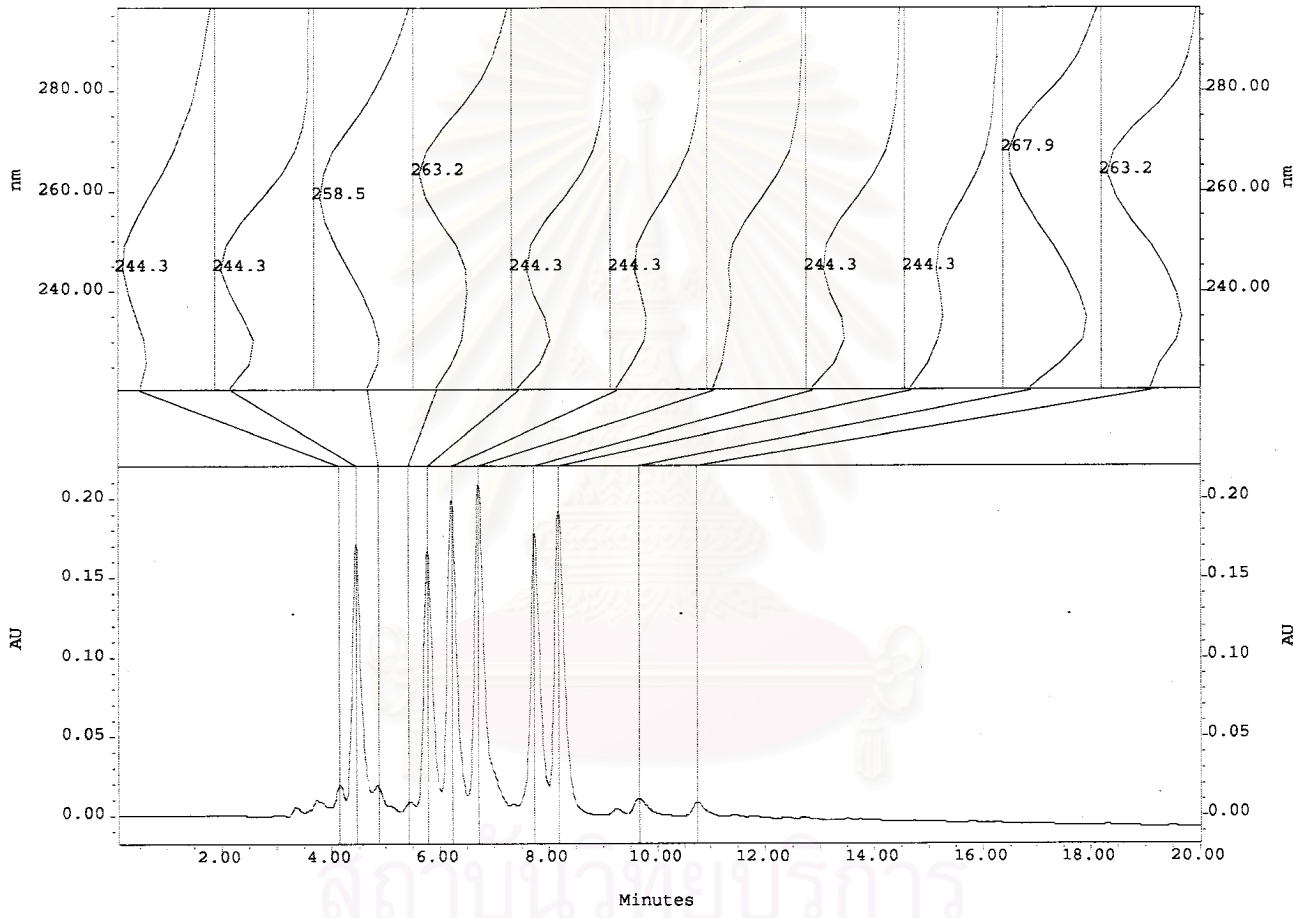

Figure 7: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and acetone (sub-libraries **4**)

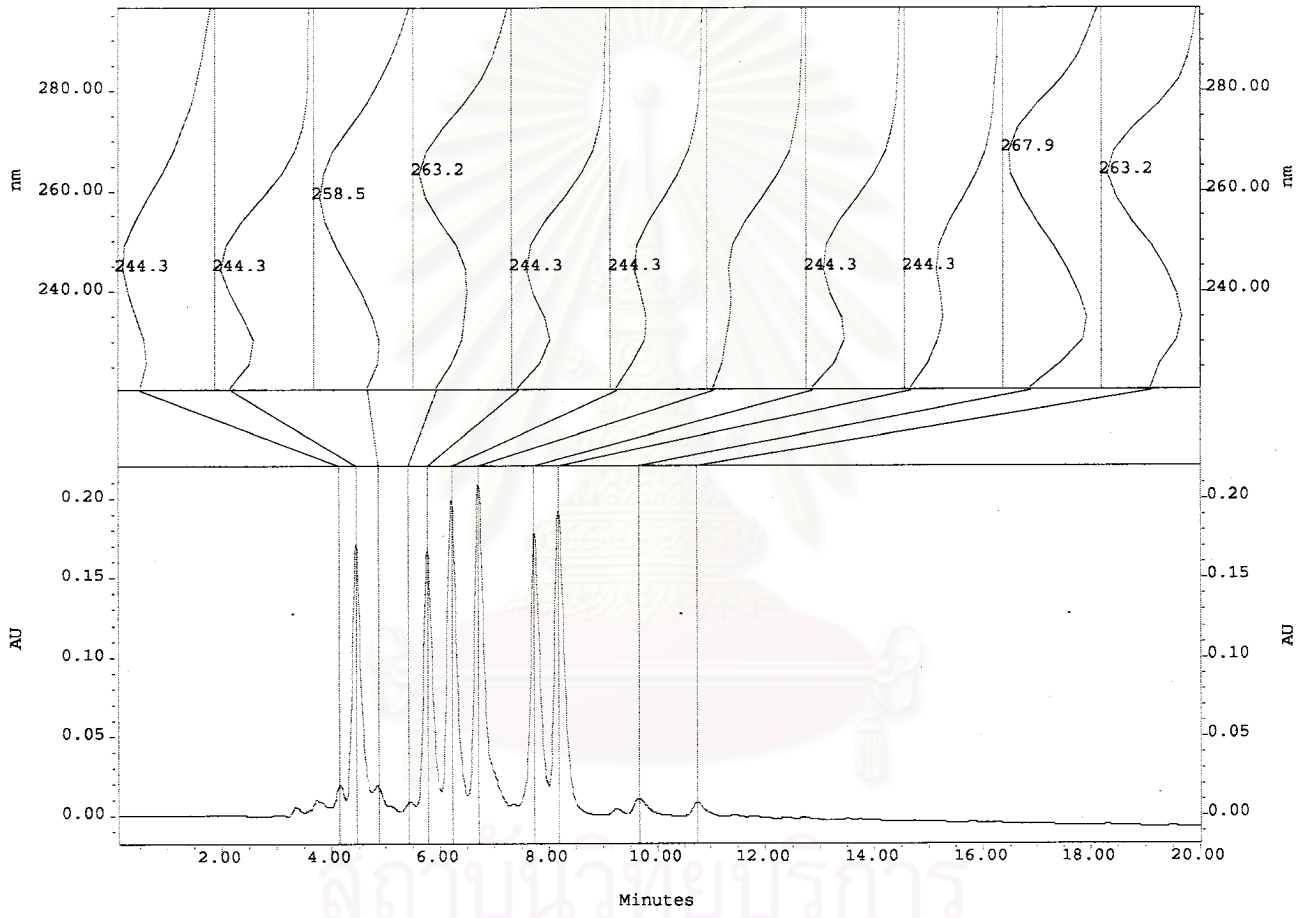

Figure 8: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and 2-pentanone (sub-libraries **5**)

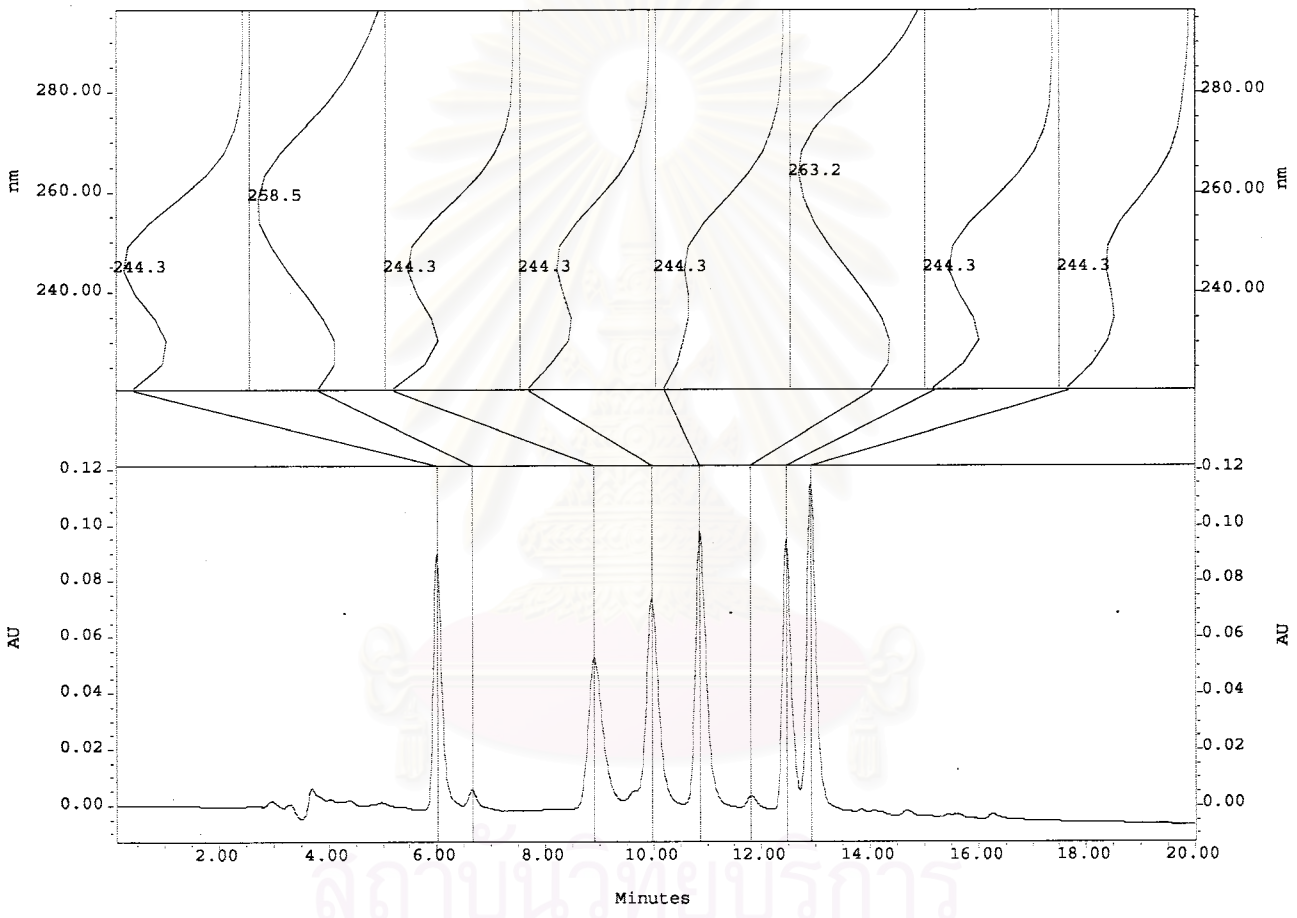

Figure 9: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and 5-methyl-2-hexanone (sub-libraries **6**)

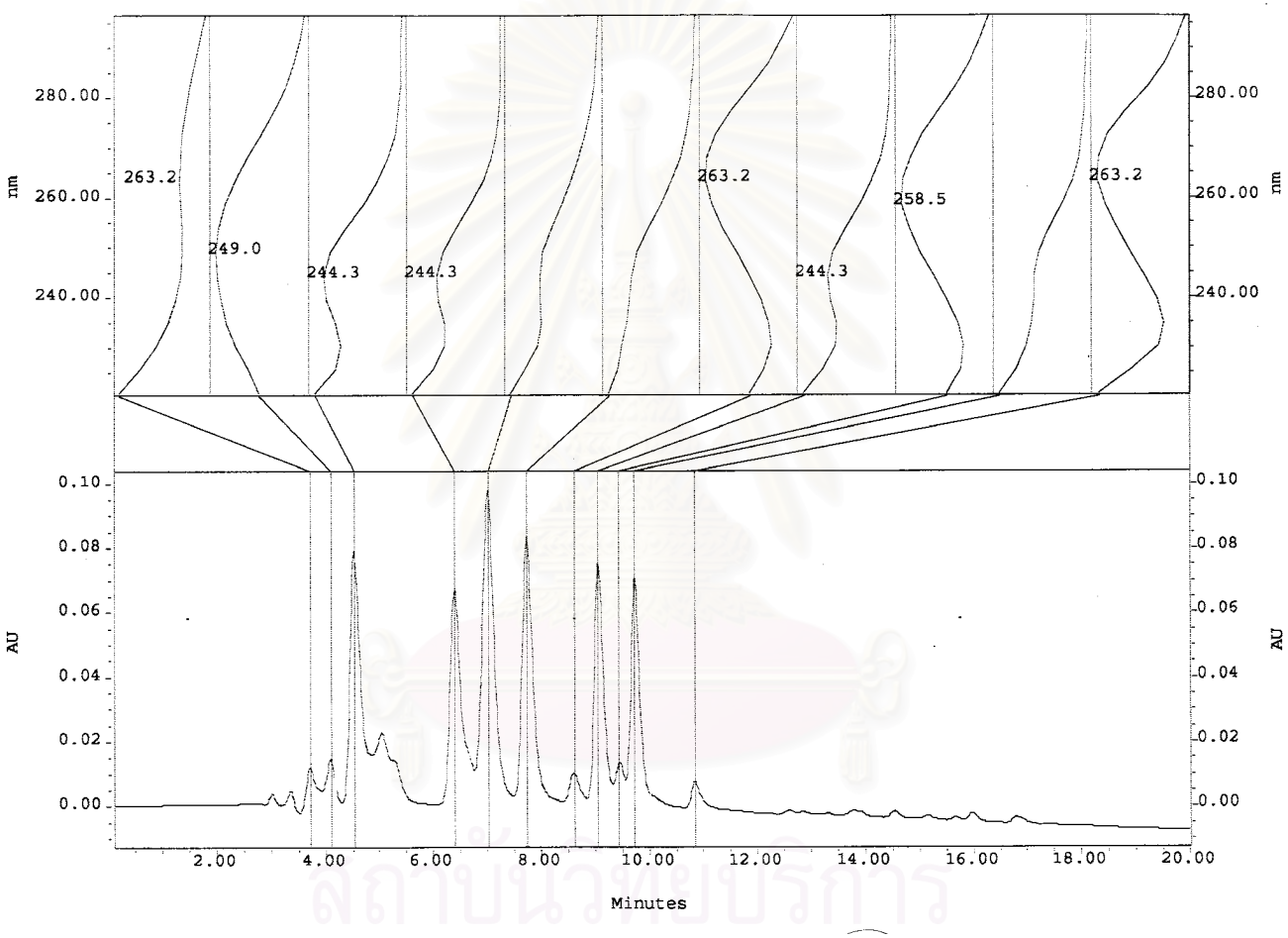

Figure 10: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and cyclopentanone (sub-libraries **7**)

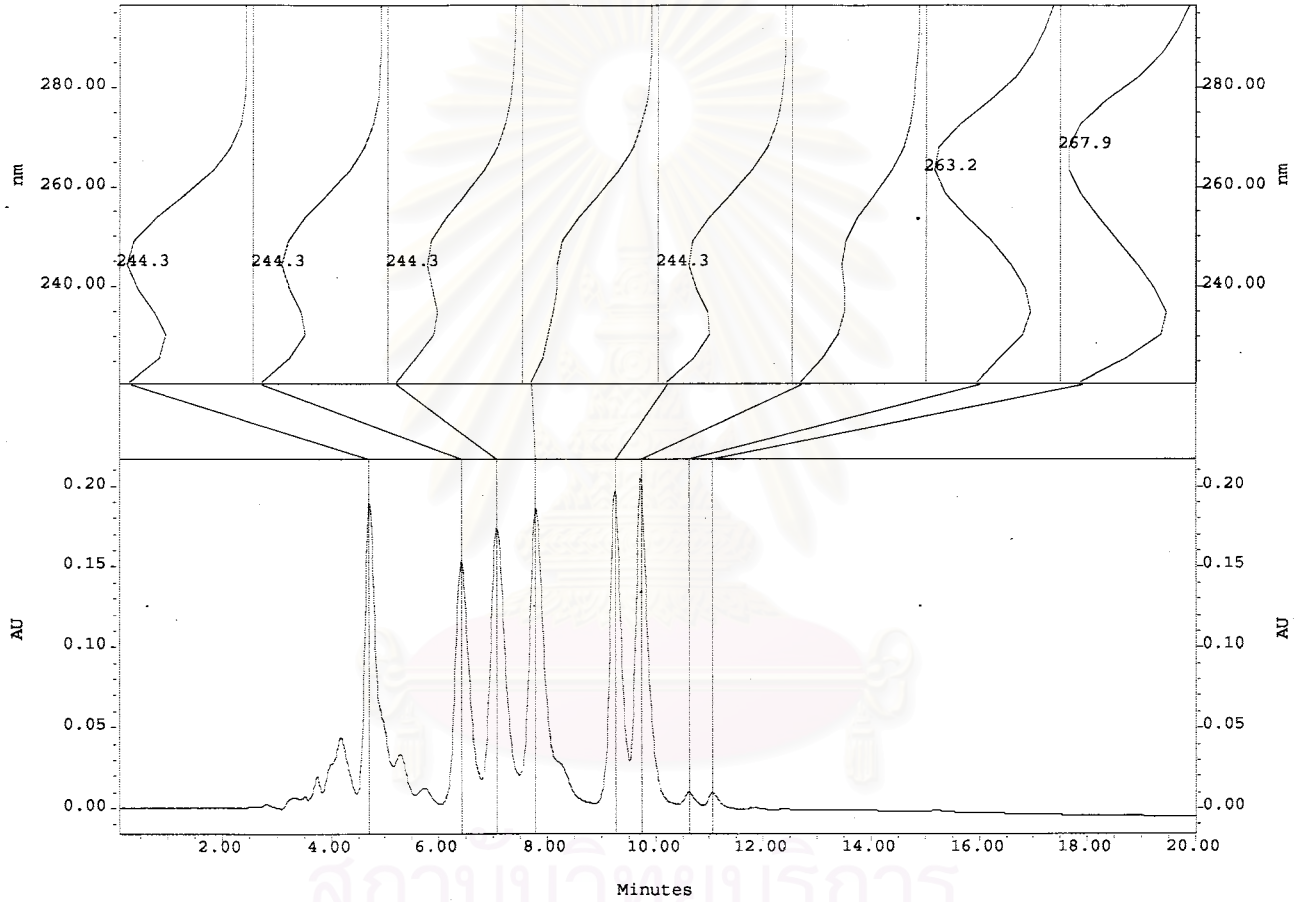

Figure 11: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and methyl isobutyl ketone (sub-libraries **8**)

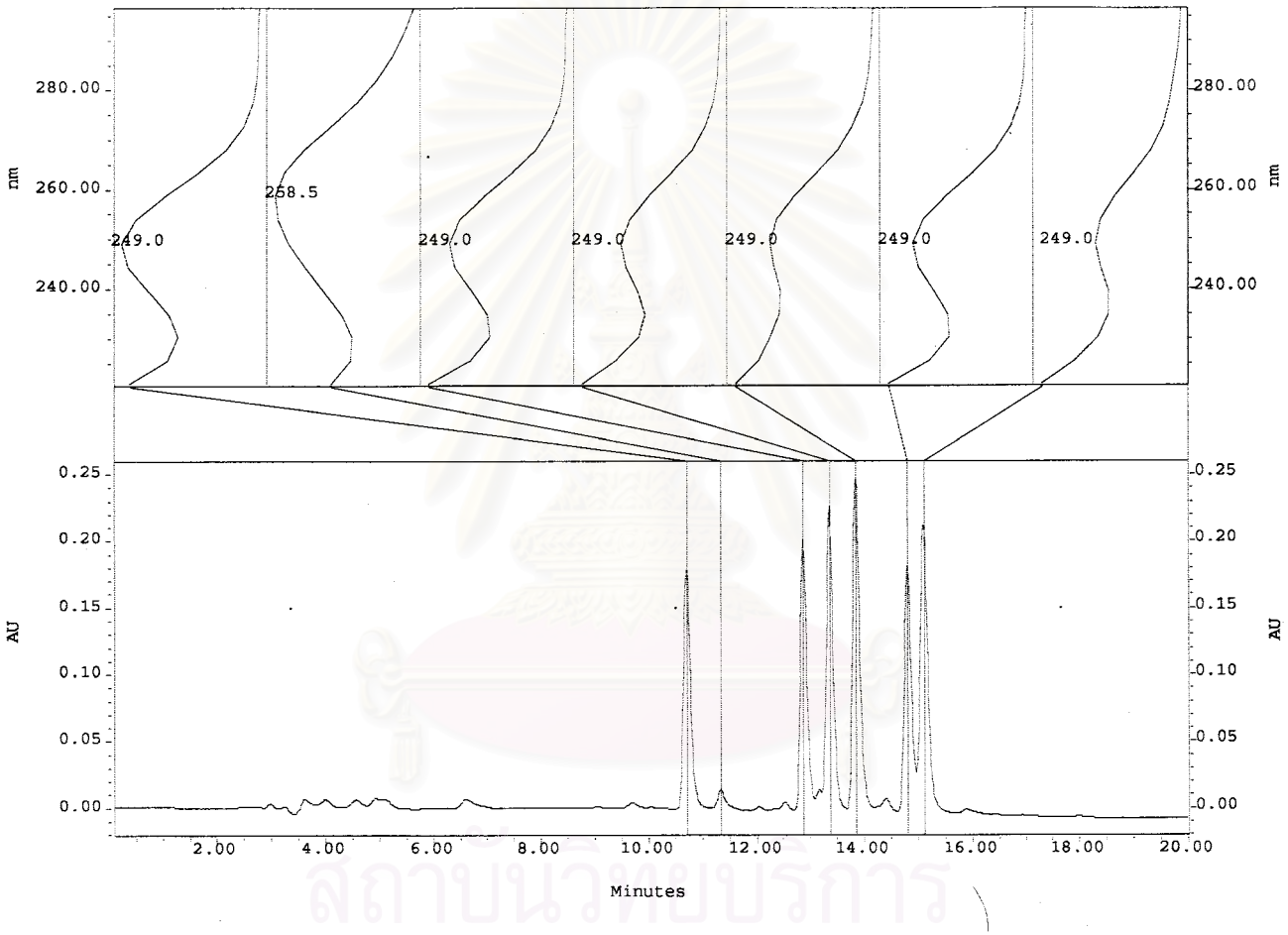

Figure 12: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and 3-octanone (sub-libraries **9**)

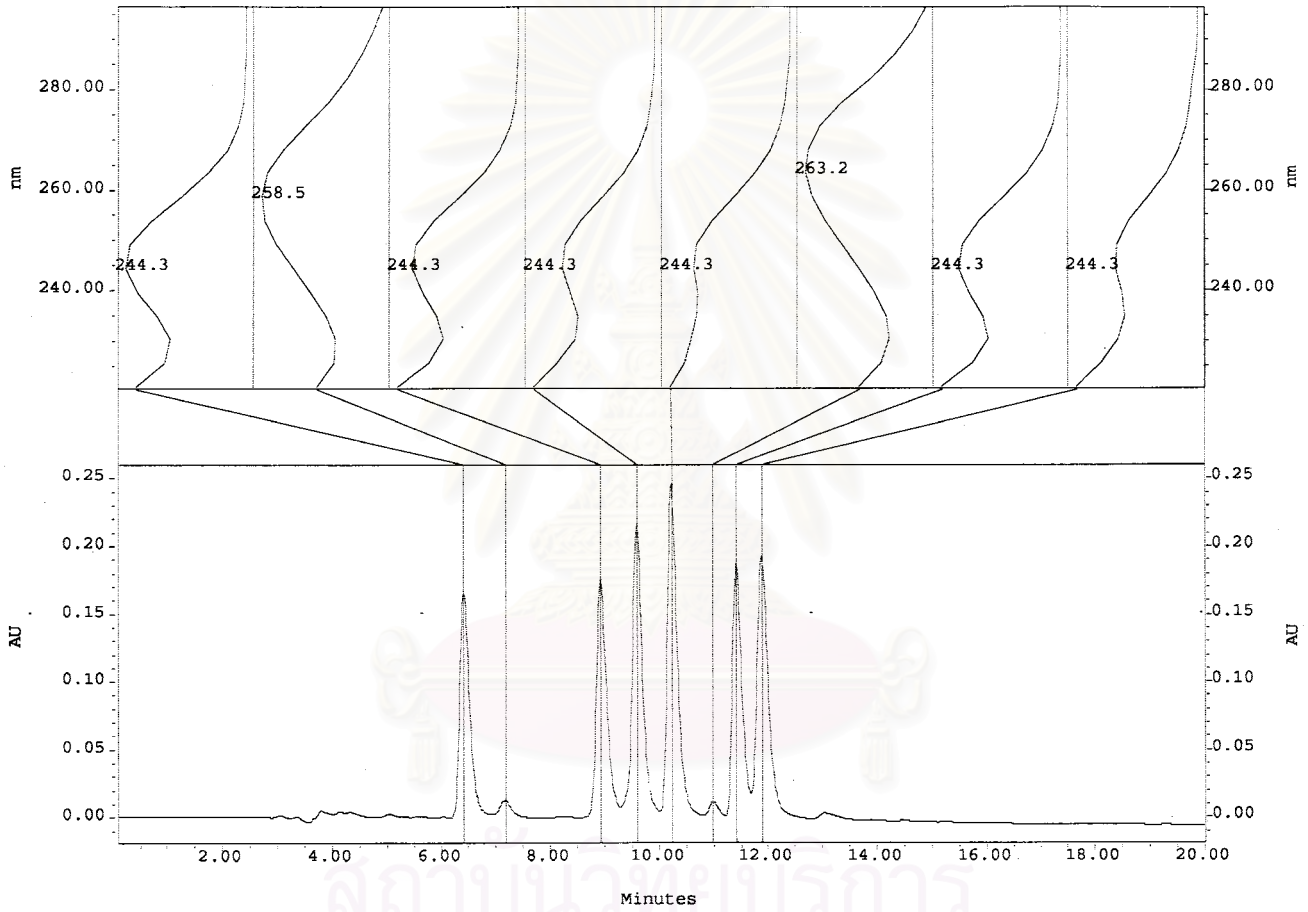

Figure 13: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and 2-heptanone (sub-libraries **10**)

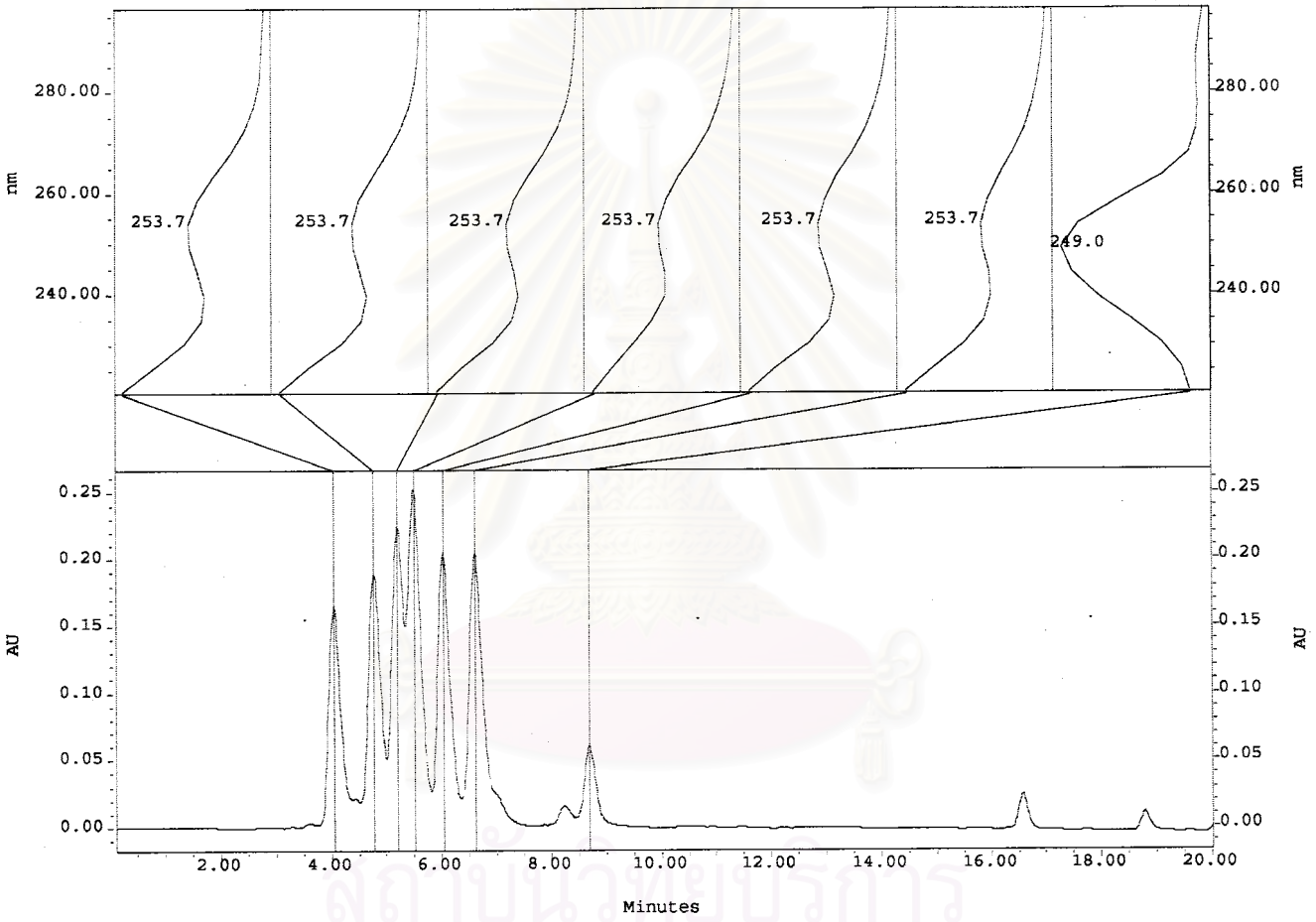

Figure 14: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and benzaldehyde (sub-libraries **11**)

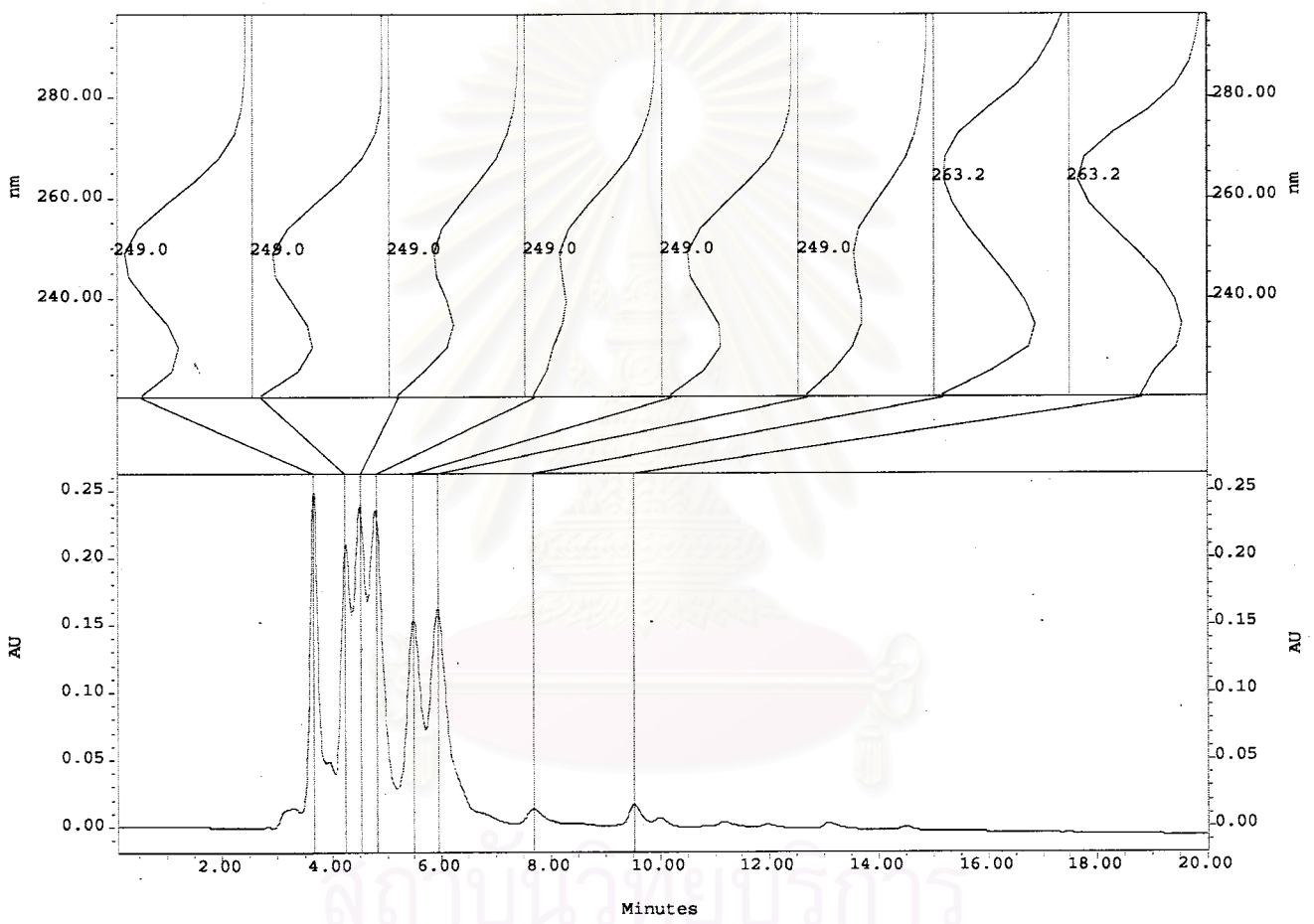

Figure 15: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and 3-pentanone (sub-libraries **12**)

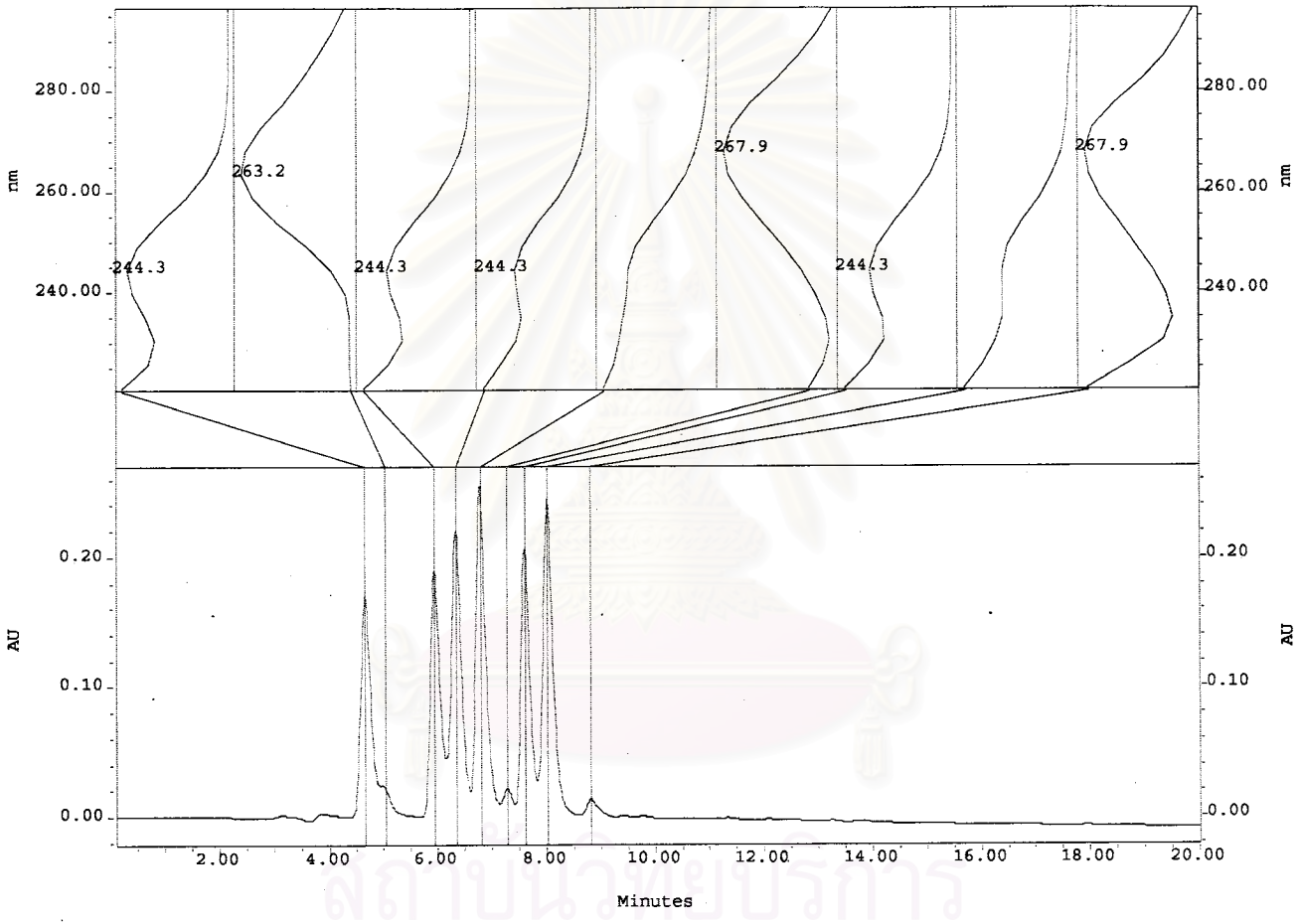

Figure 16: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and cyclohexanone (sub-libraries **13**)

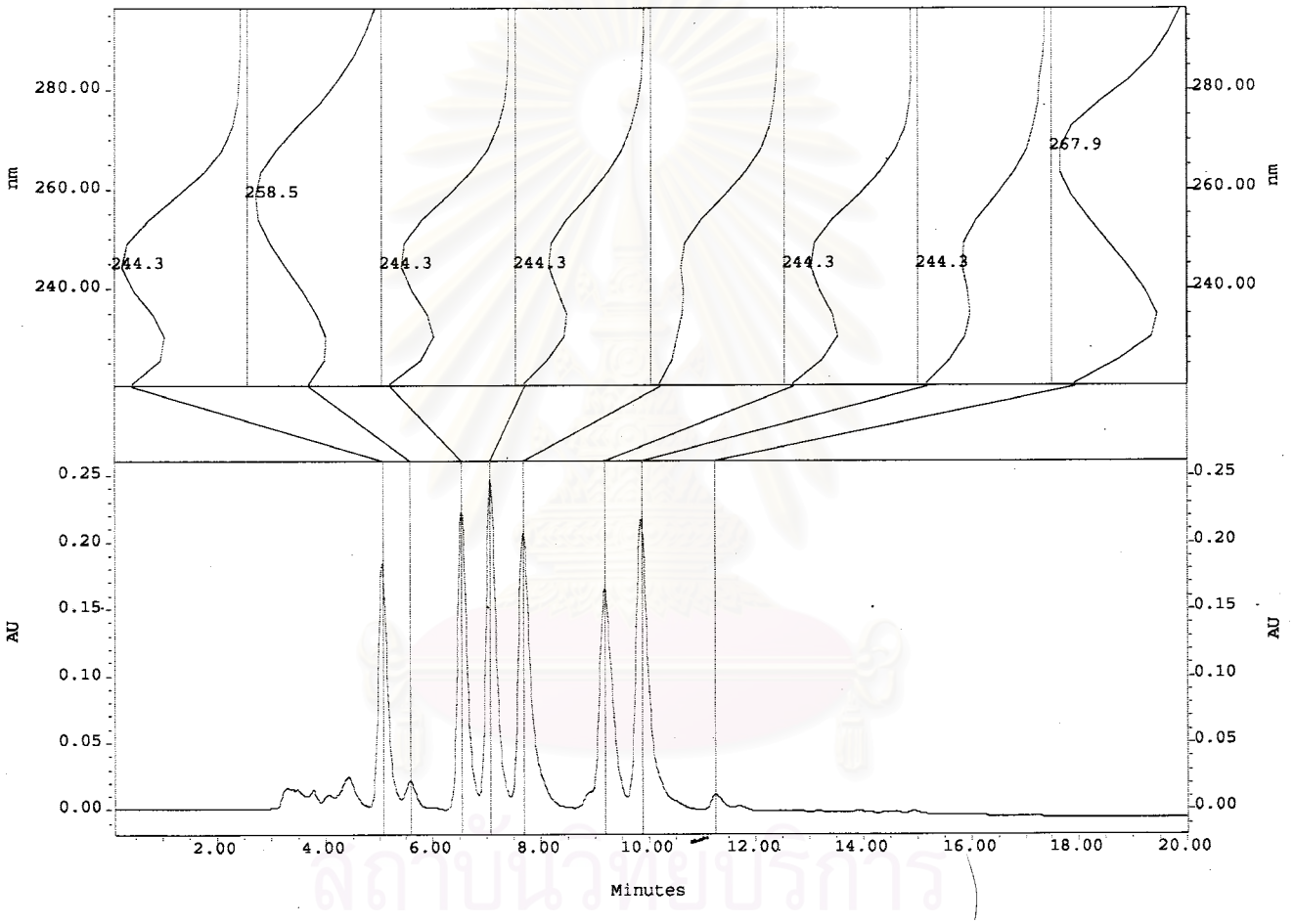

Figure 17: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and 2-hexanone (sub-libraries **14**)

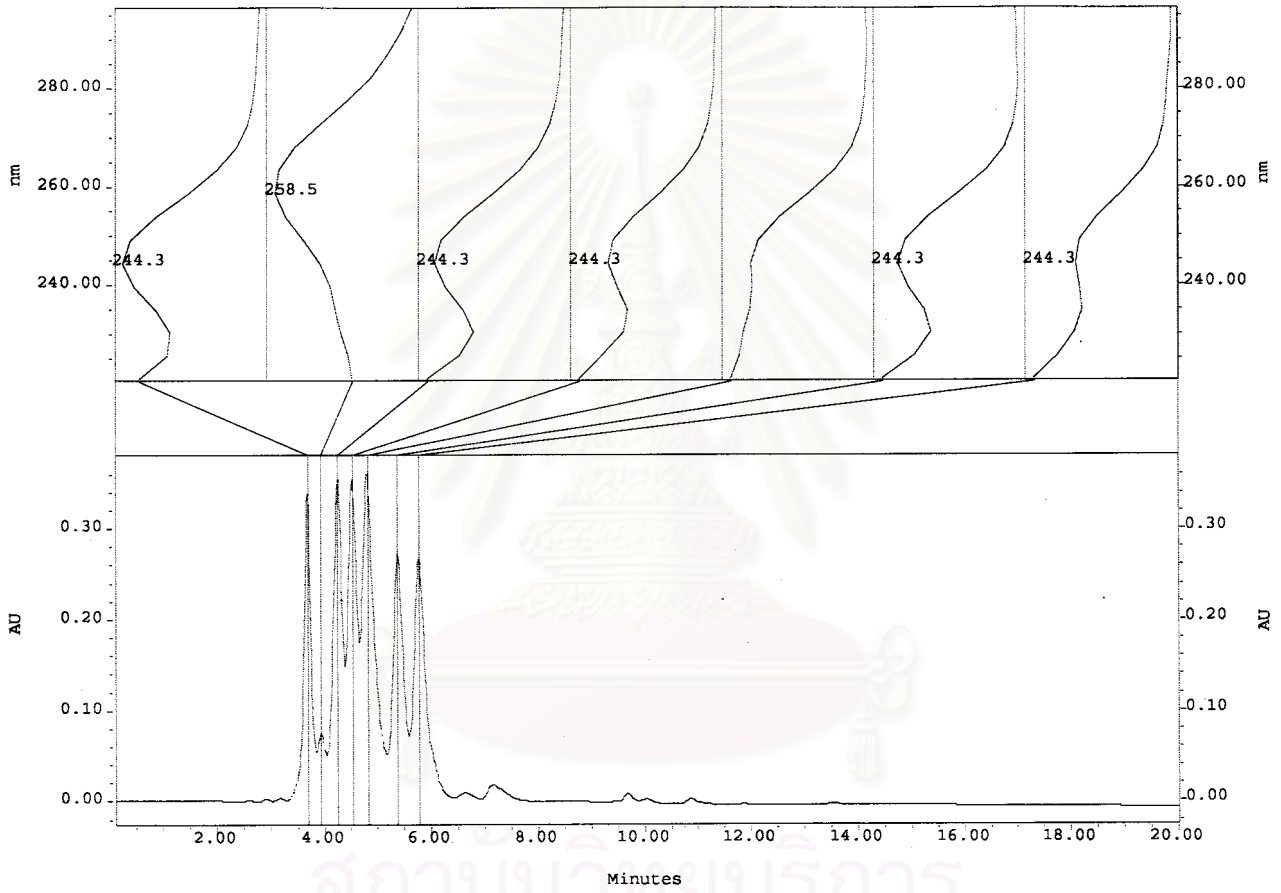

Figure 18: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and 2-butanone (sub-libraries **15**)

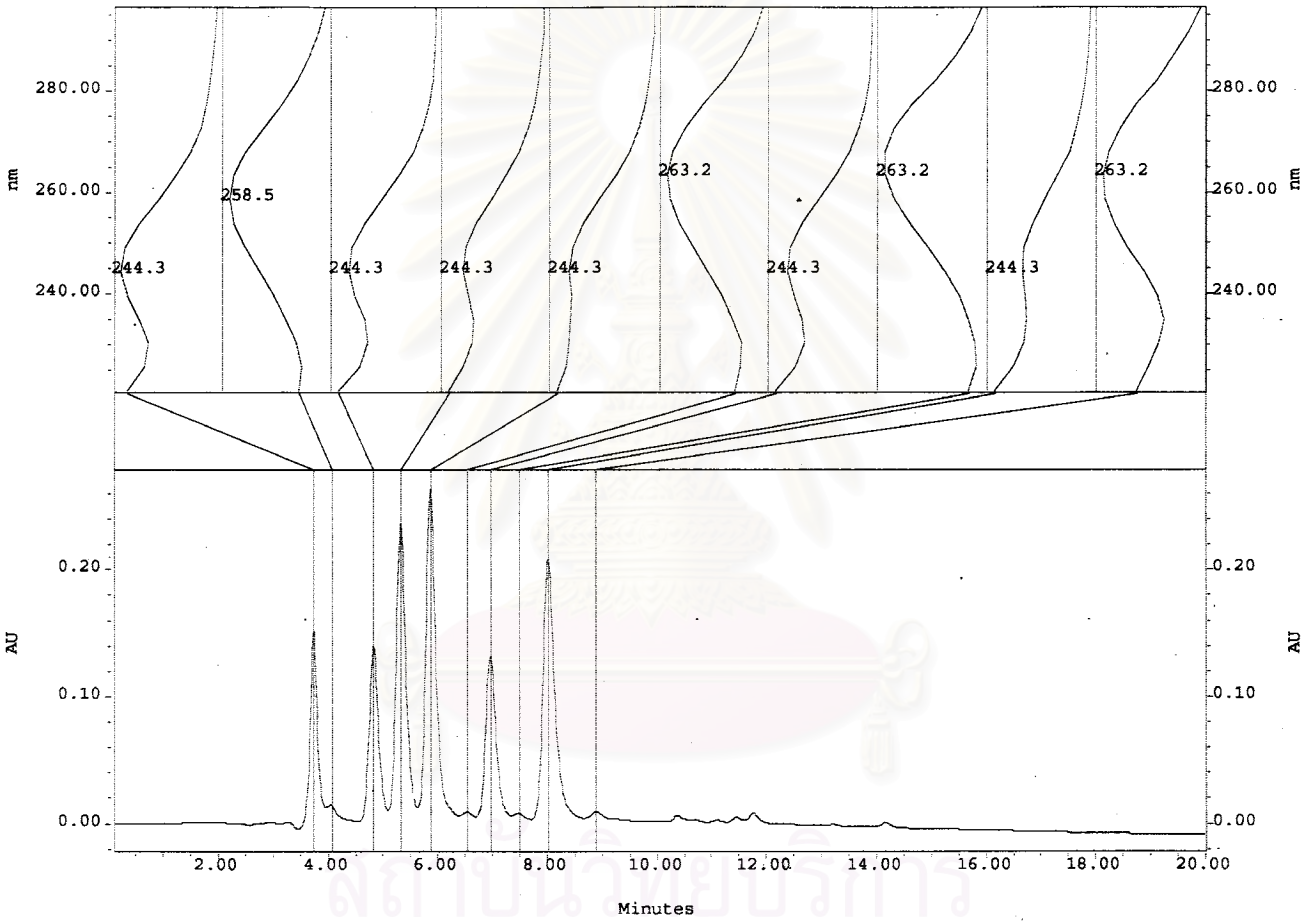

Figure 19: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and acetaldehyde (sub-libraries **16**)

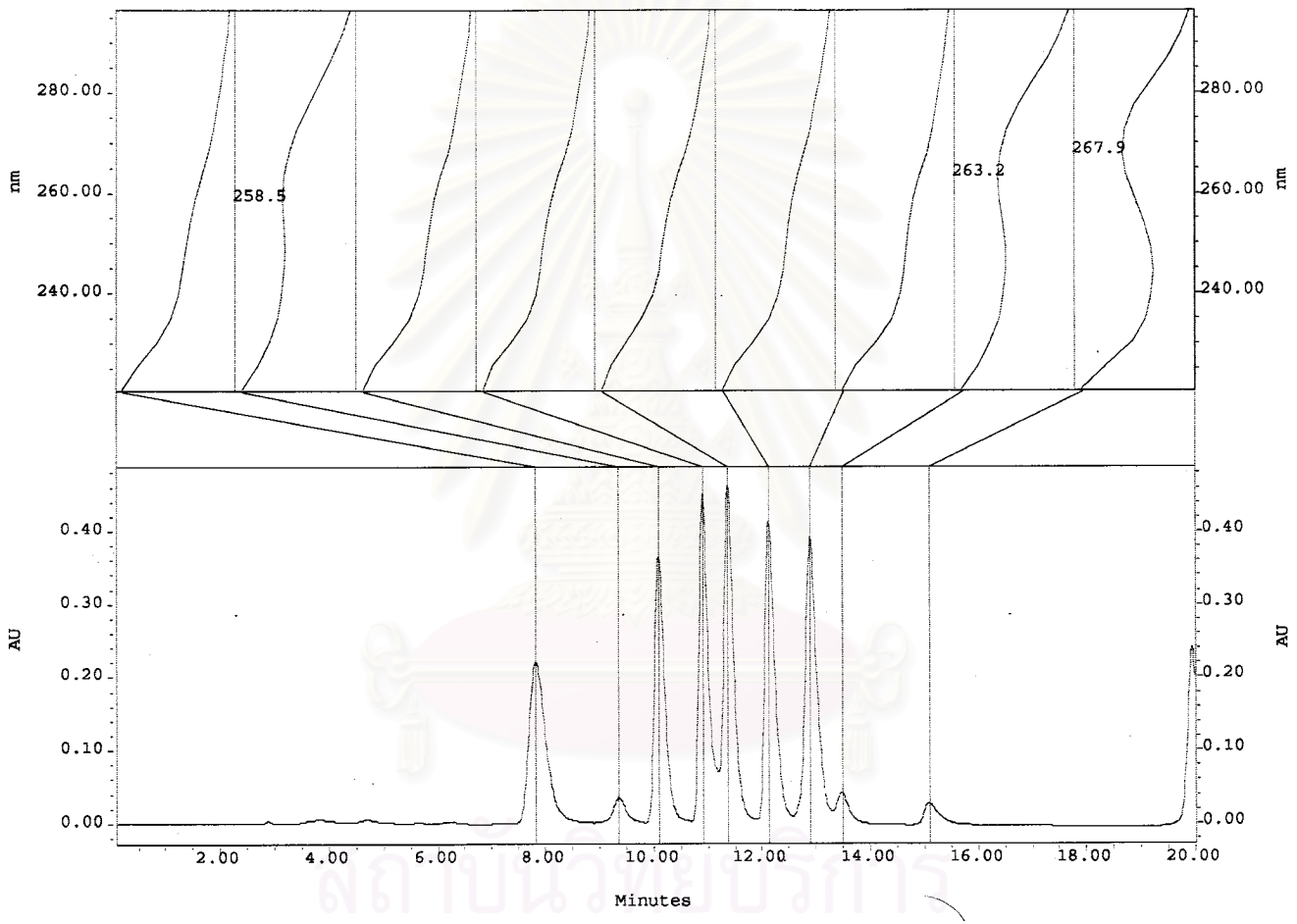

Figure 20: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and 3-phenoxybenzaldehyde (sub-libraries **17**)

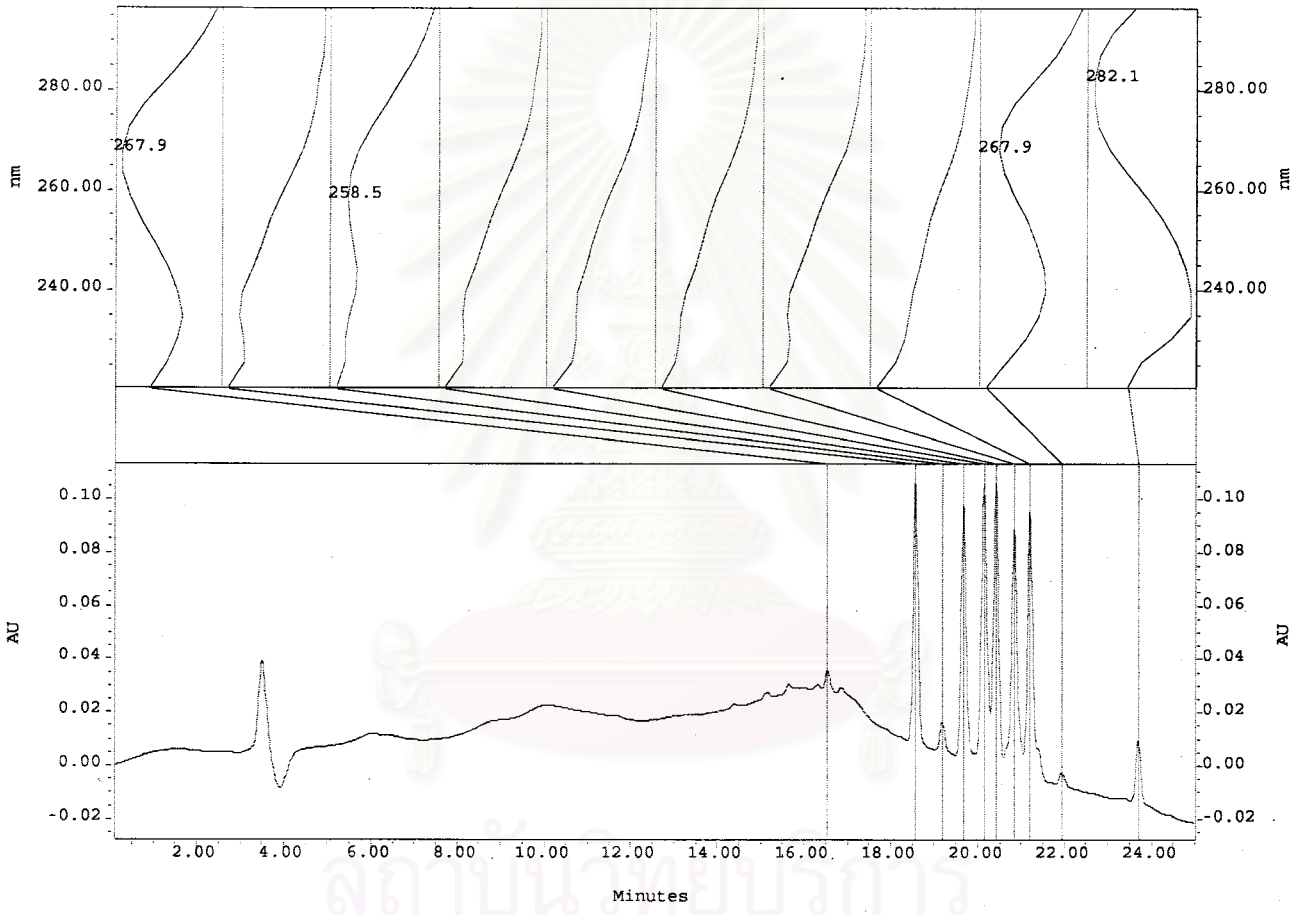

Figure 21: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and 4-benzyloxybenzaldehyde (sub-libraries **18**)

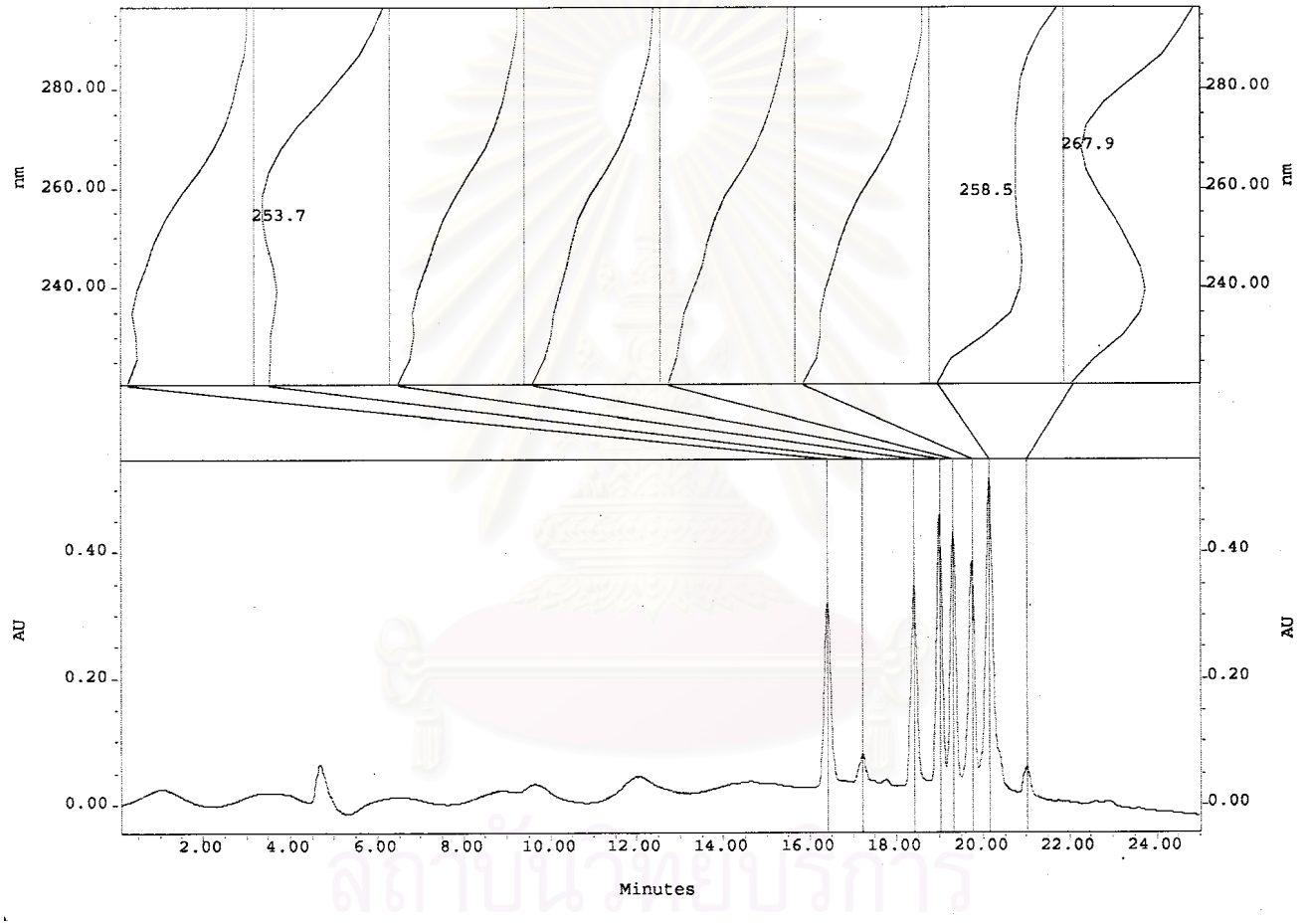

Figure 22: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and 4-methoxybezaldehyde (sub-libraries **19**)

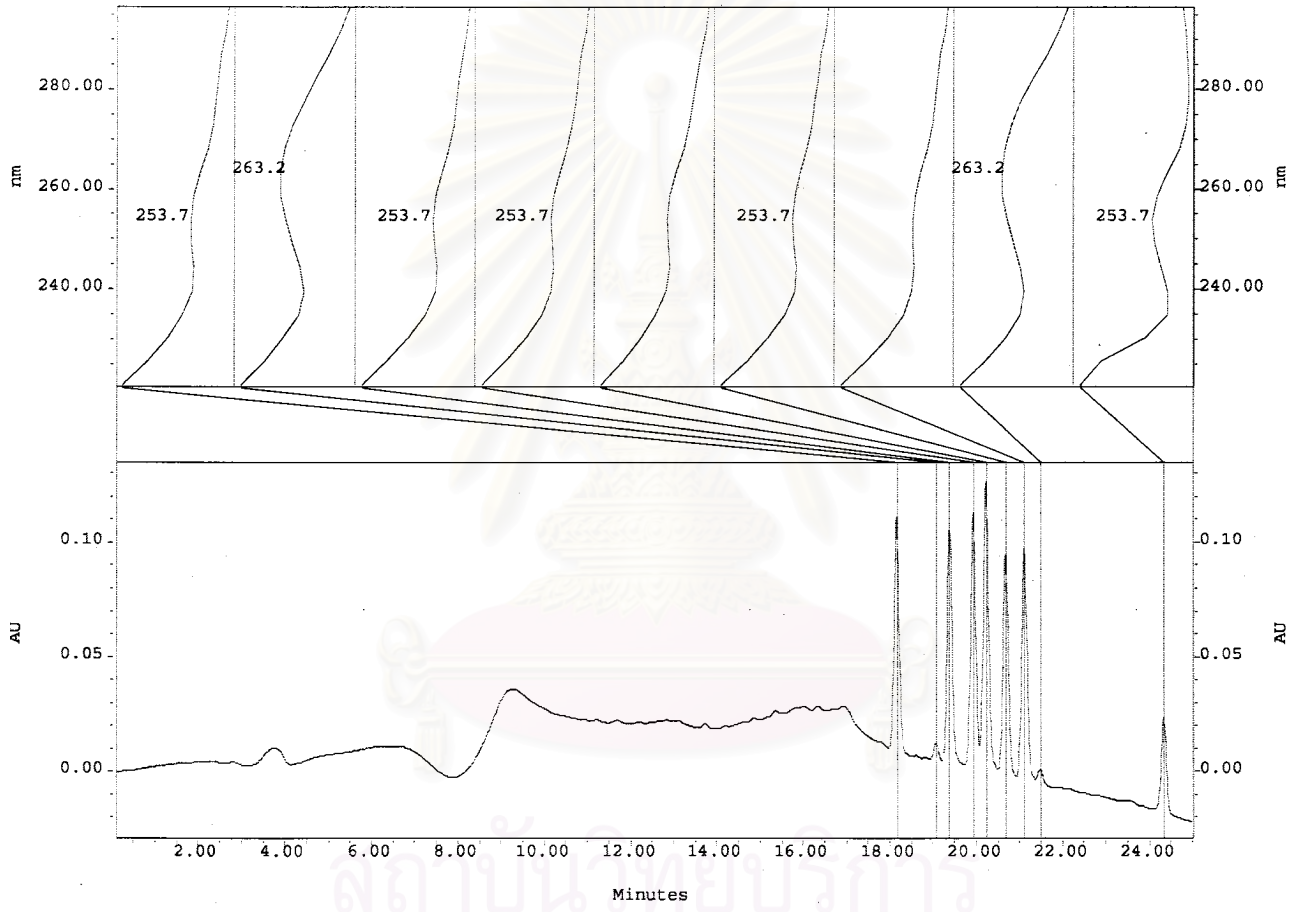

Figure 23: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of arylbiguanide hydrochloride and 3-benzyloxybenzaldehyde (sub-libraries **20**)

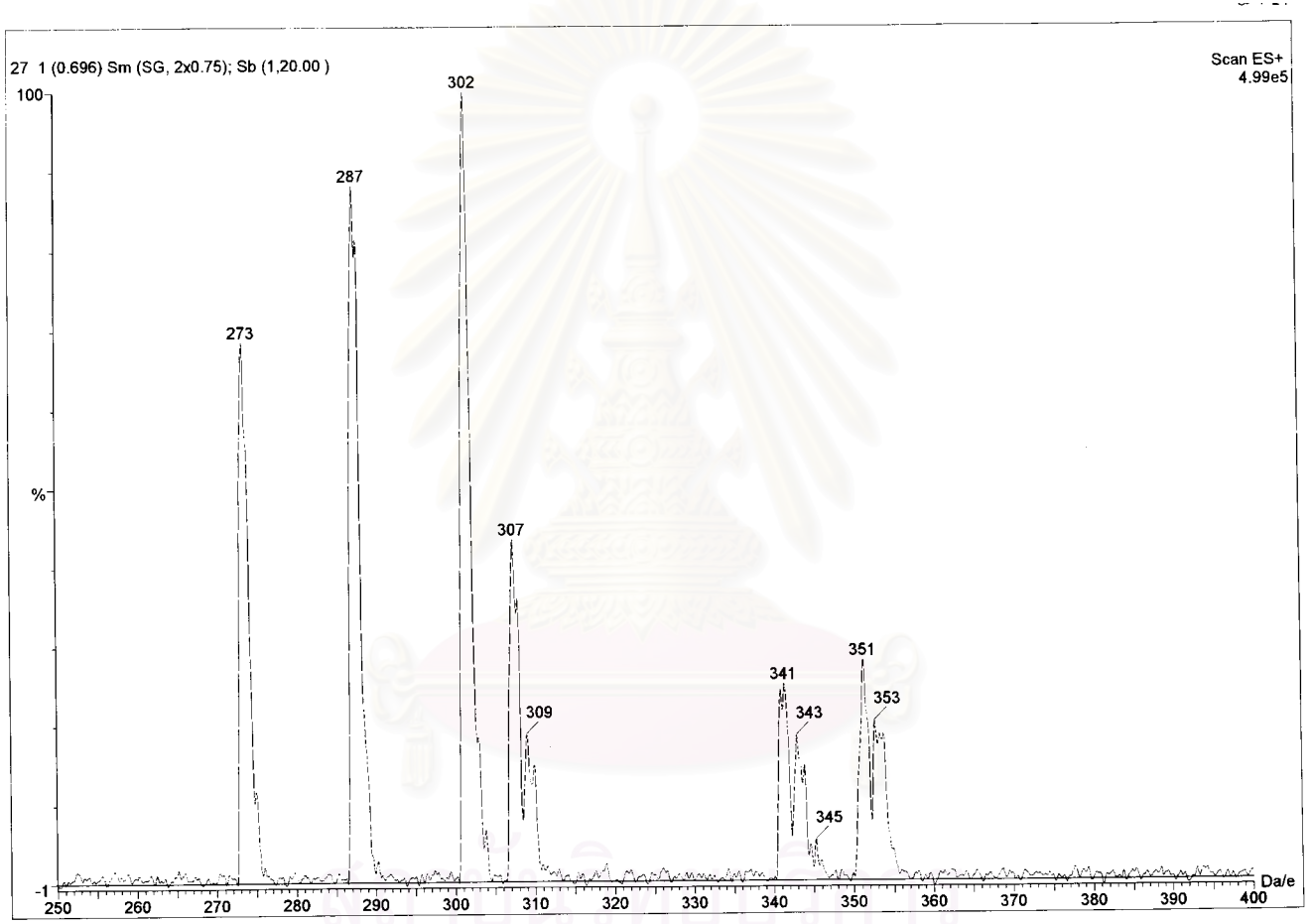

Figure 24: ESI mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and 4-heptanone (sub-libraries **1**)

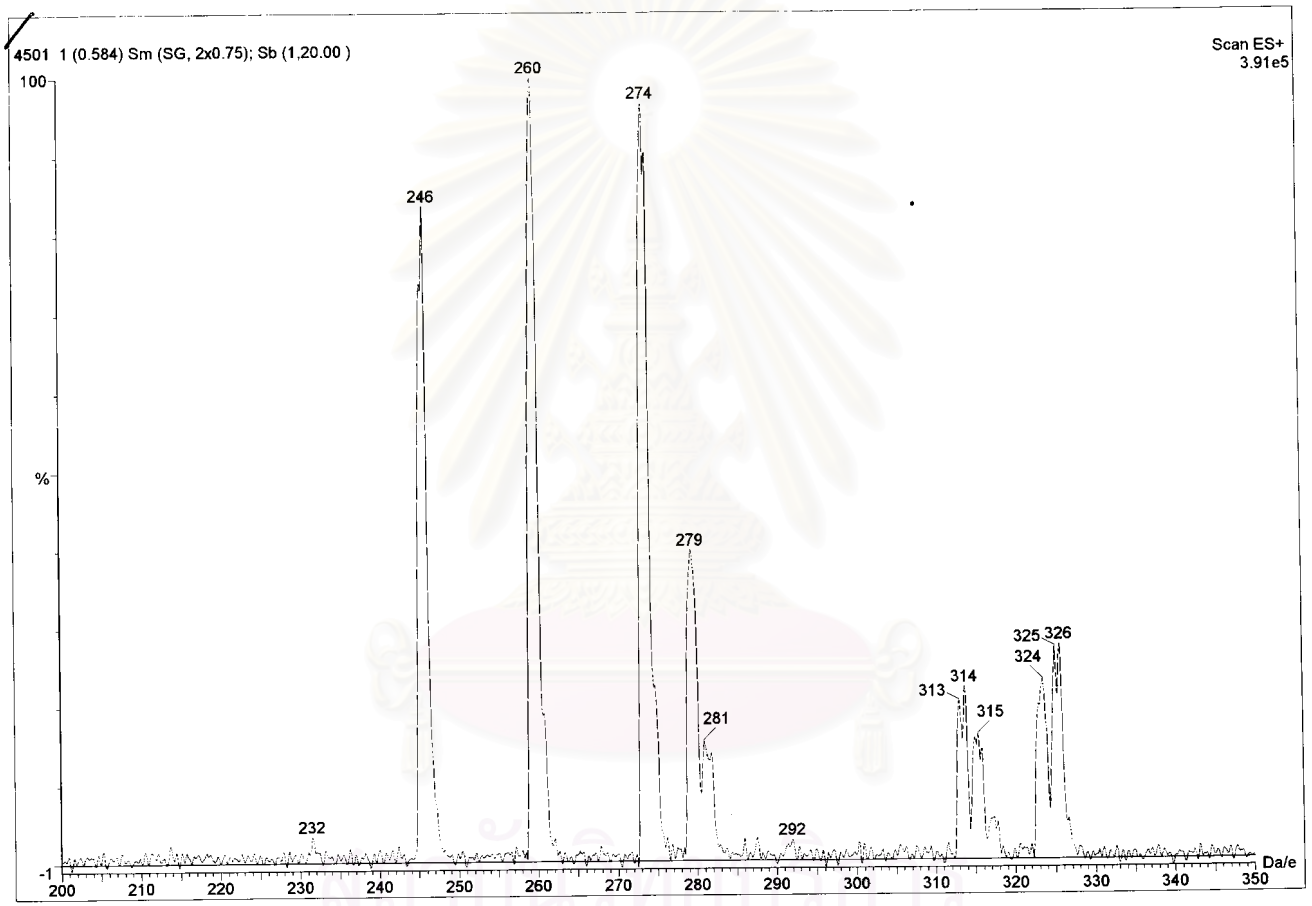

Figure 25: ESI mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and 3-methyl-2-butanone (sub-libraries **2**)

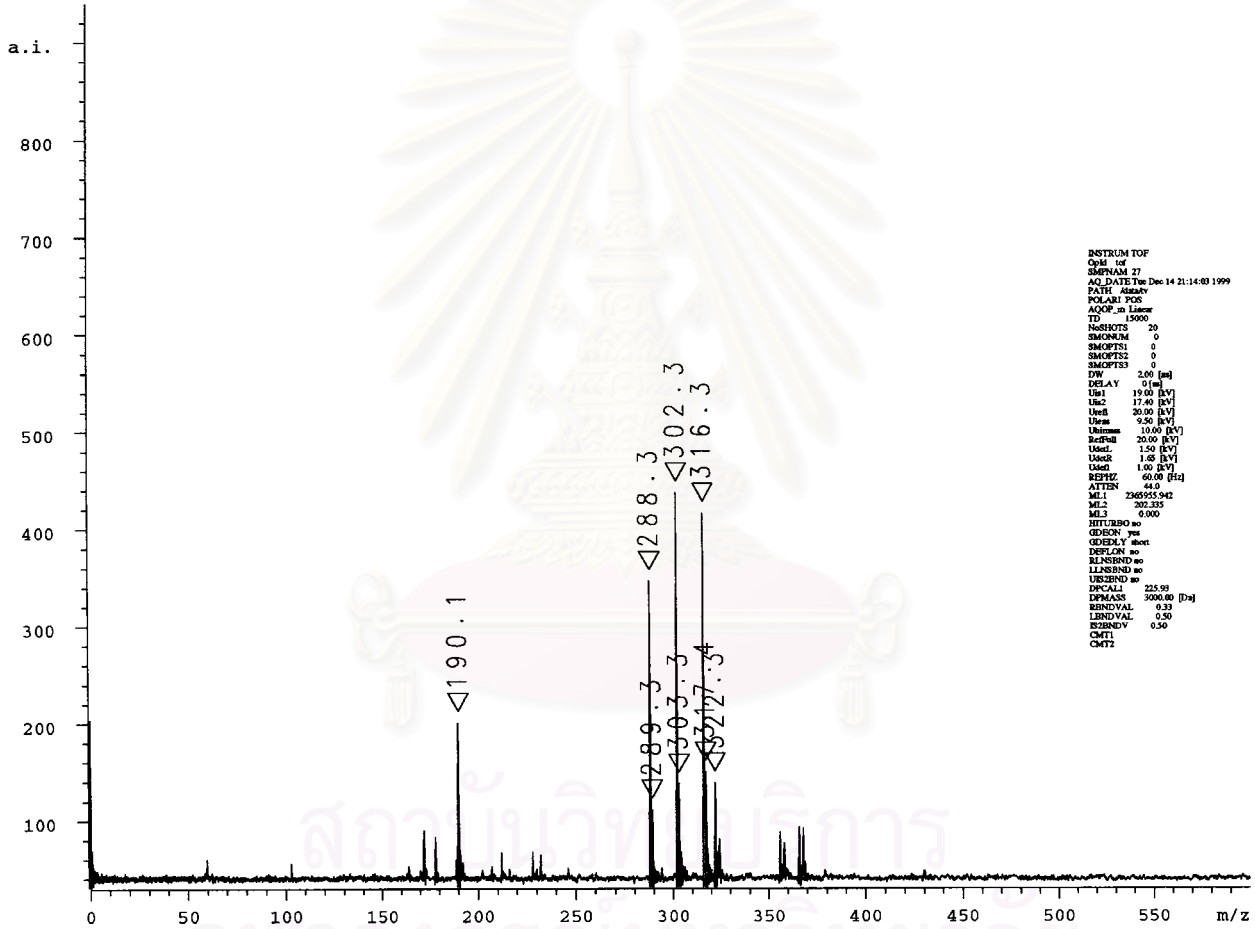

Figure 26: MALDI-TOF mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and 2-octanone (sub-libraries **3**)

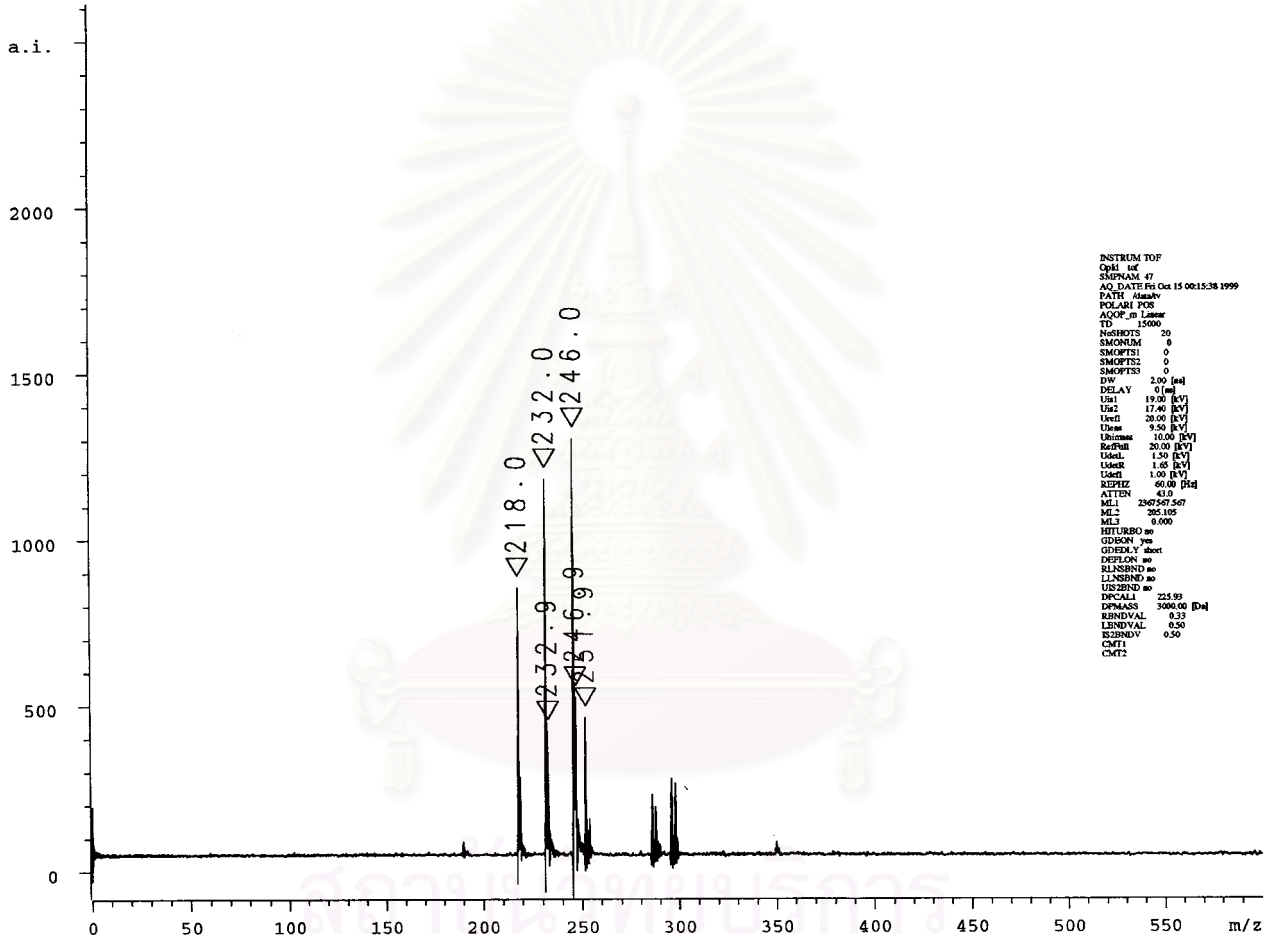

Figure 27: MALDI-TOF mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and acetone (sub-libraries **4**)

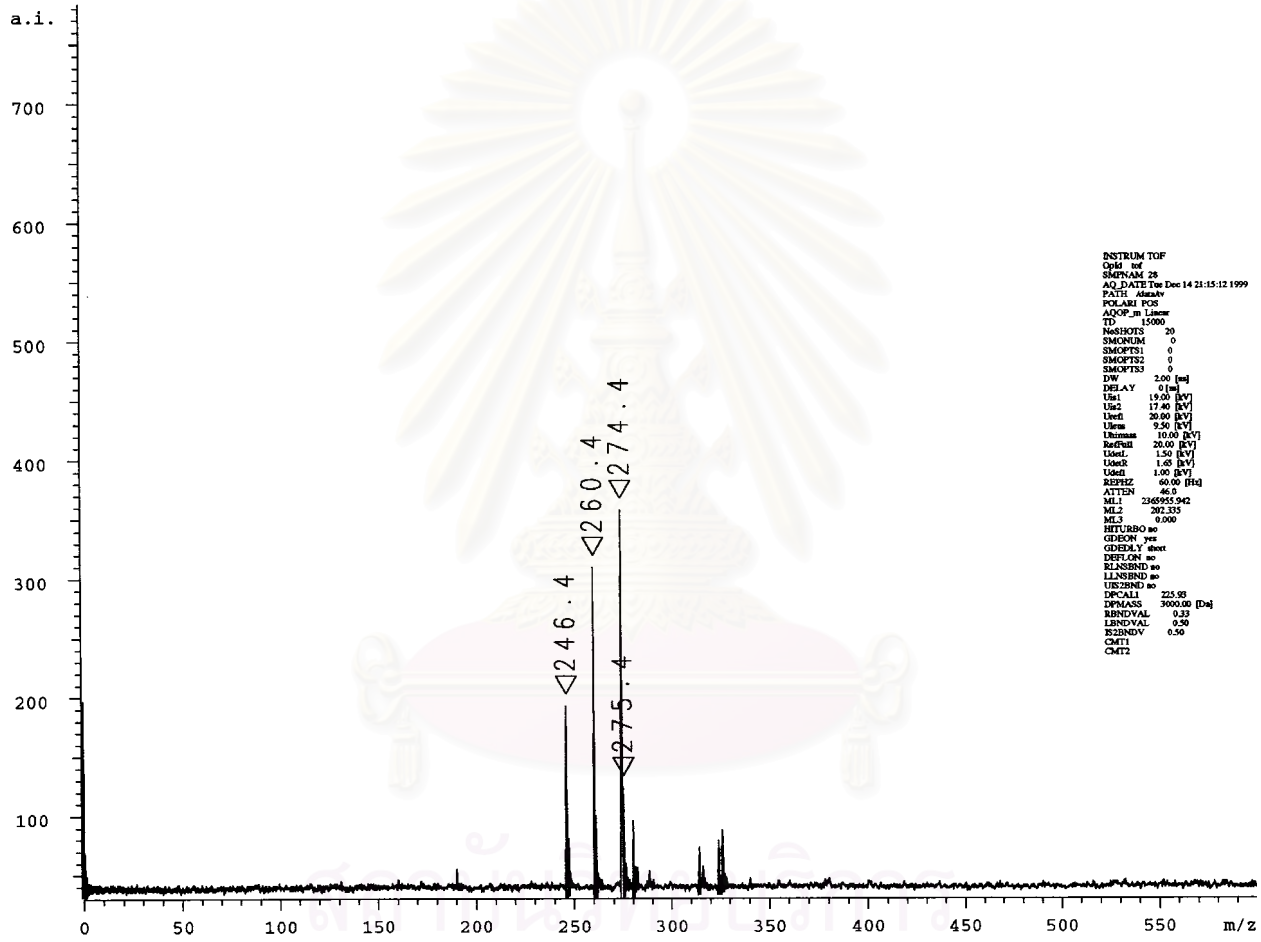

Figure 28: MALDI-TOF mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and 2-pentanone (sub-libraries **5**)

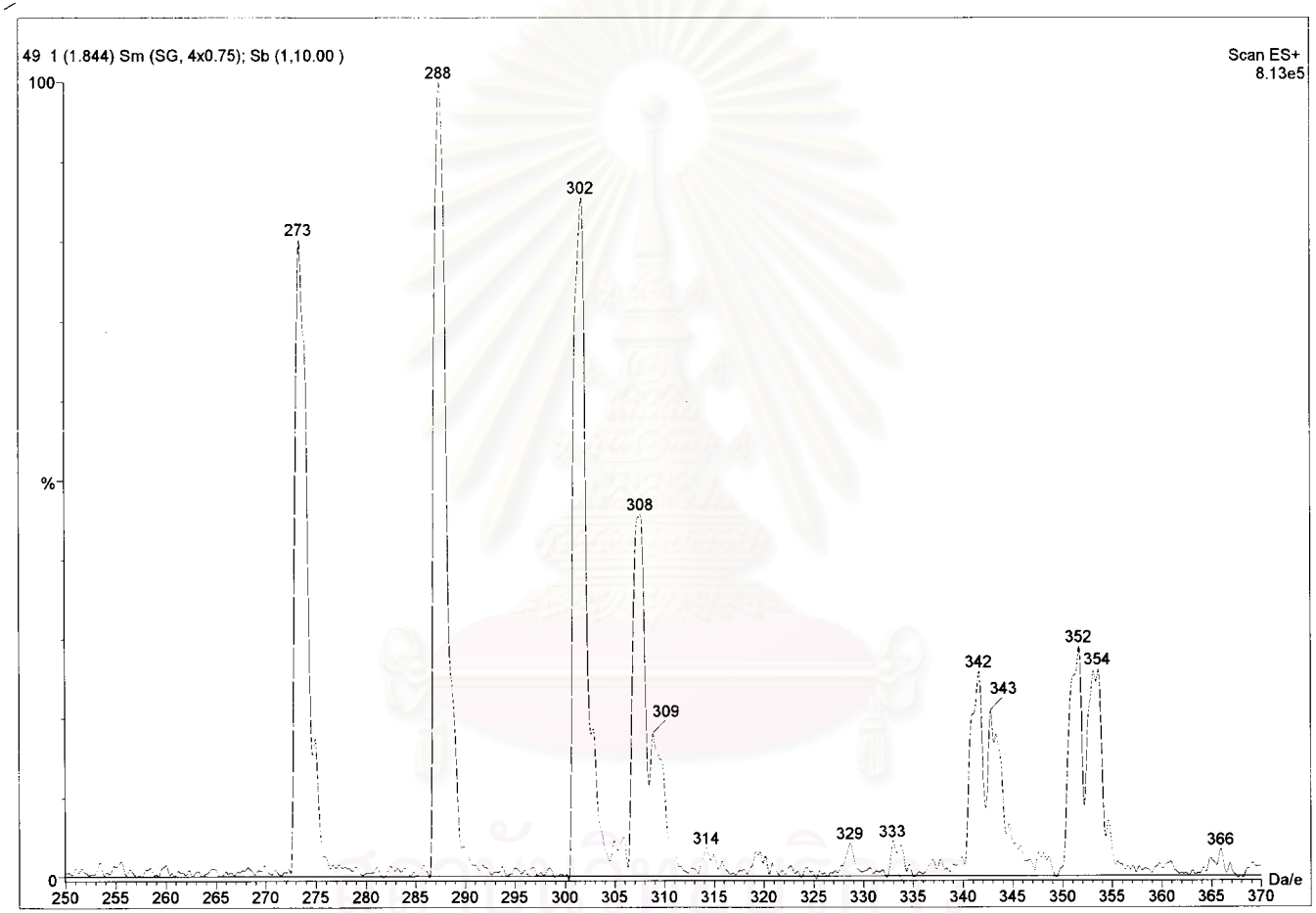

Figure 29: ESI mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and 5-methyl-2-hexanone (sub-libraries **6**)

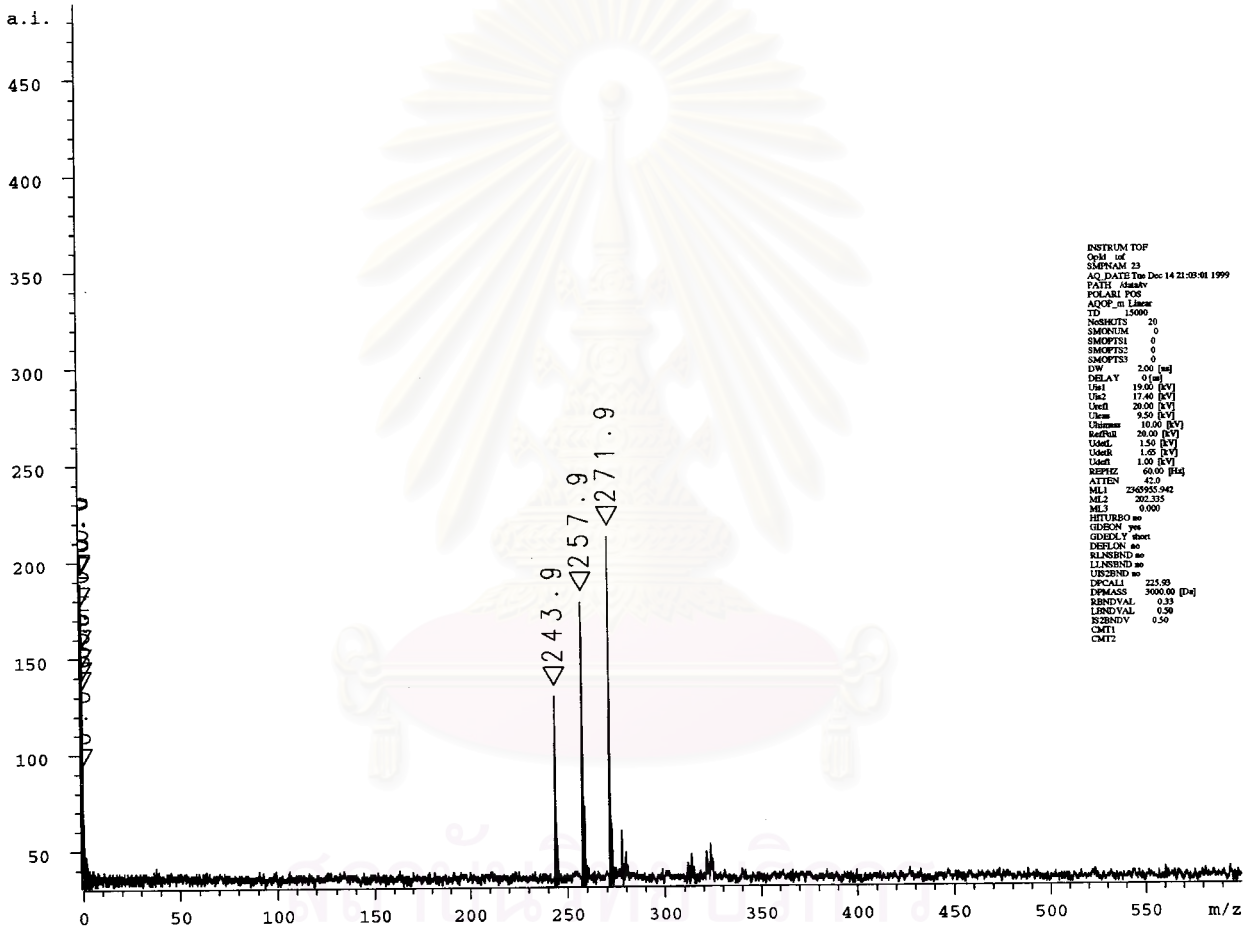

Figure 30: MALDI-TOF mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and cyclopentanone (sub-libraries **7**)

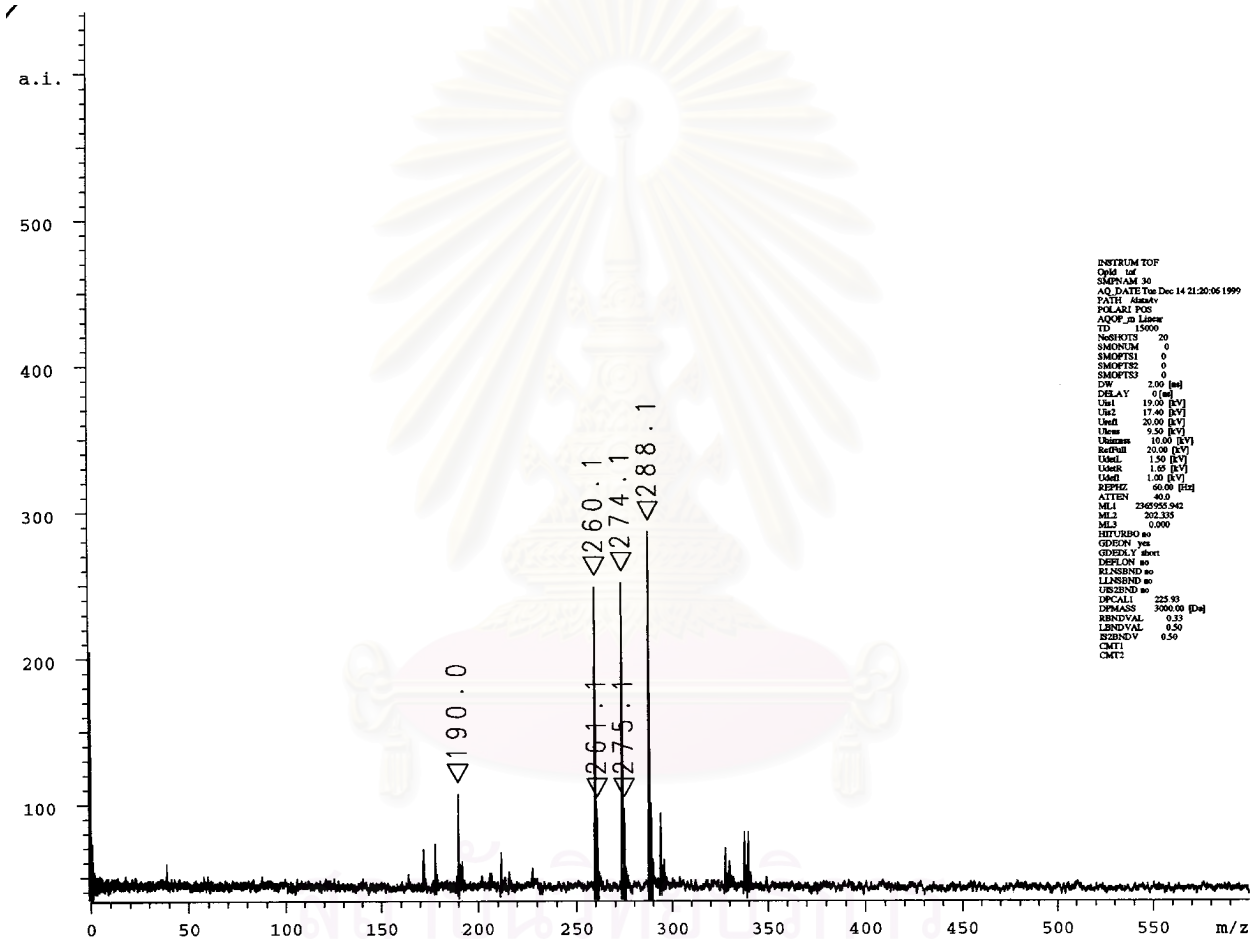

Figure 31: MALDI-TOF mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and methyl isobutyl ketone (sub-libraries **8**)

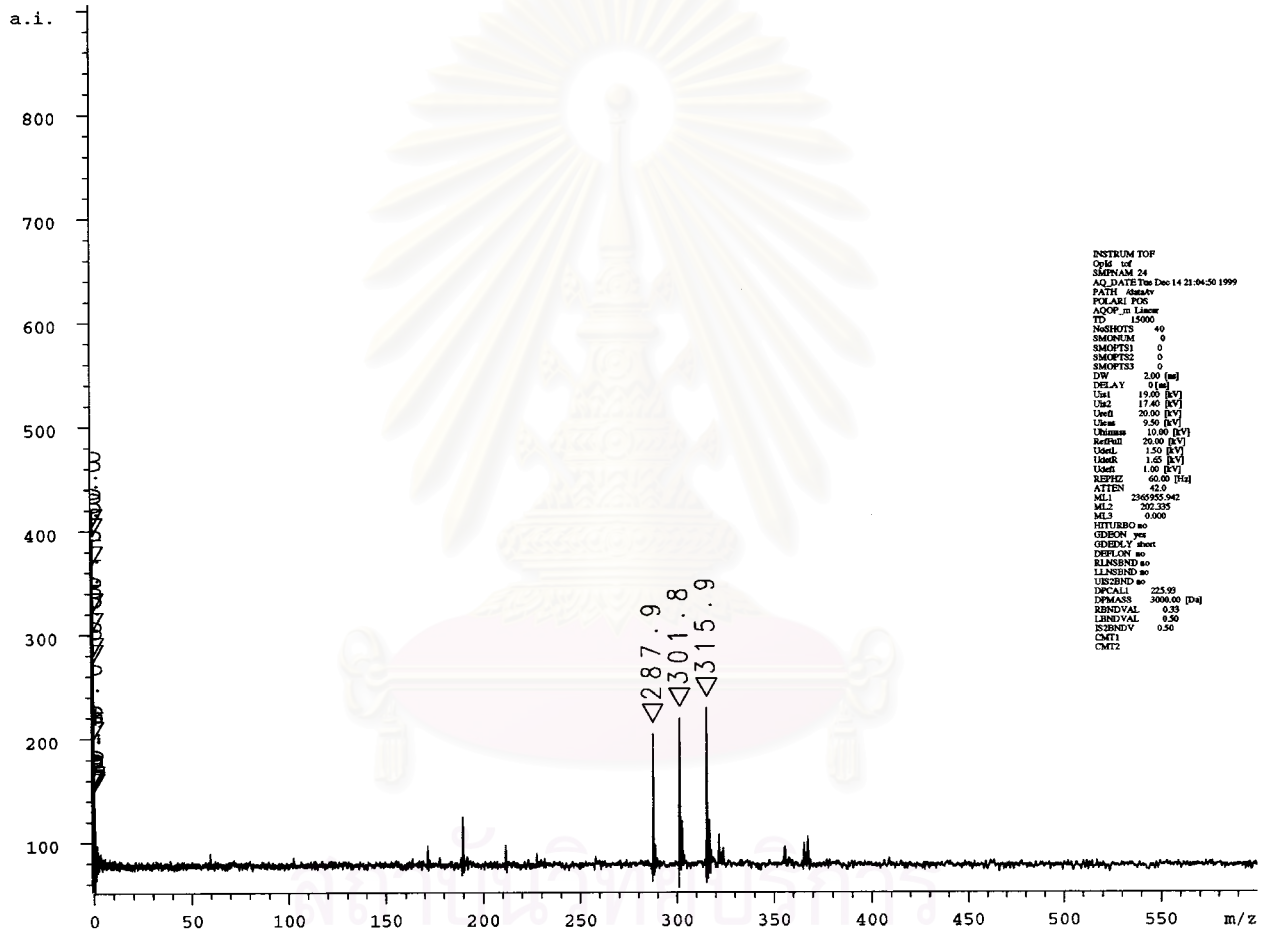

Figure 32: MALDI-TOF mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and 3-octanone (sub-libraries **9**)

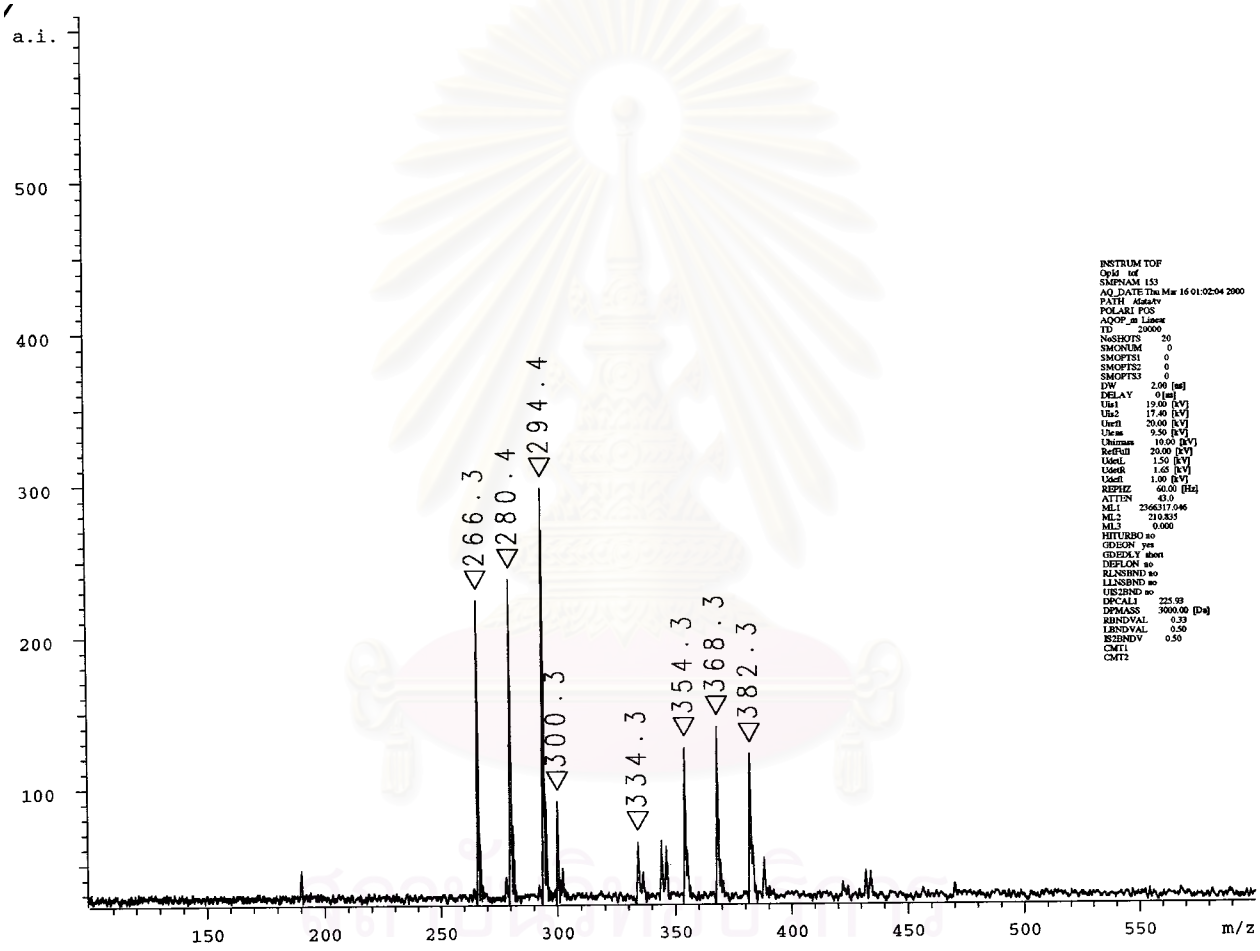

Figure 33: MALDI-TOF mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and 2-heptanone (sub-libraries **10**)

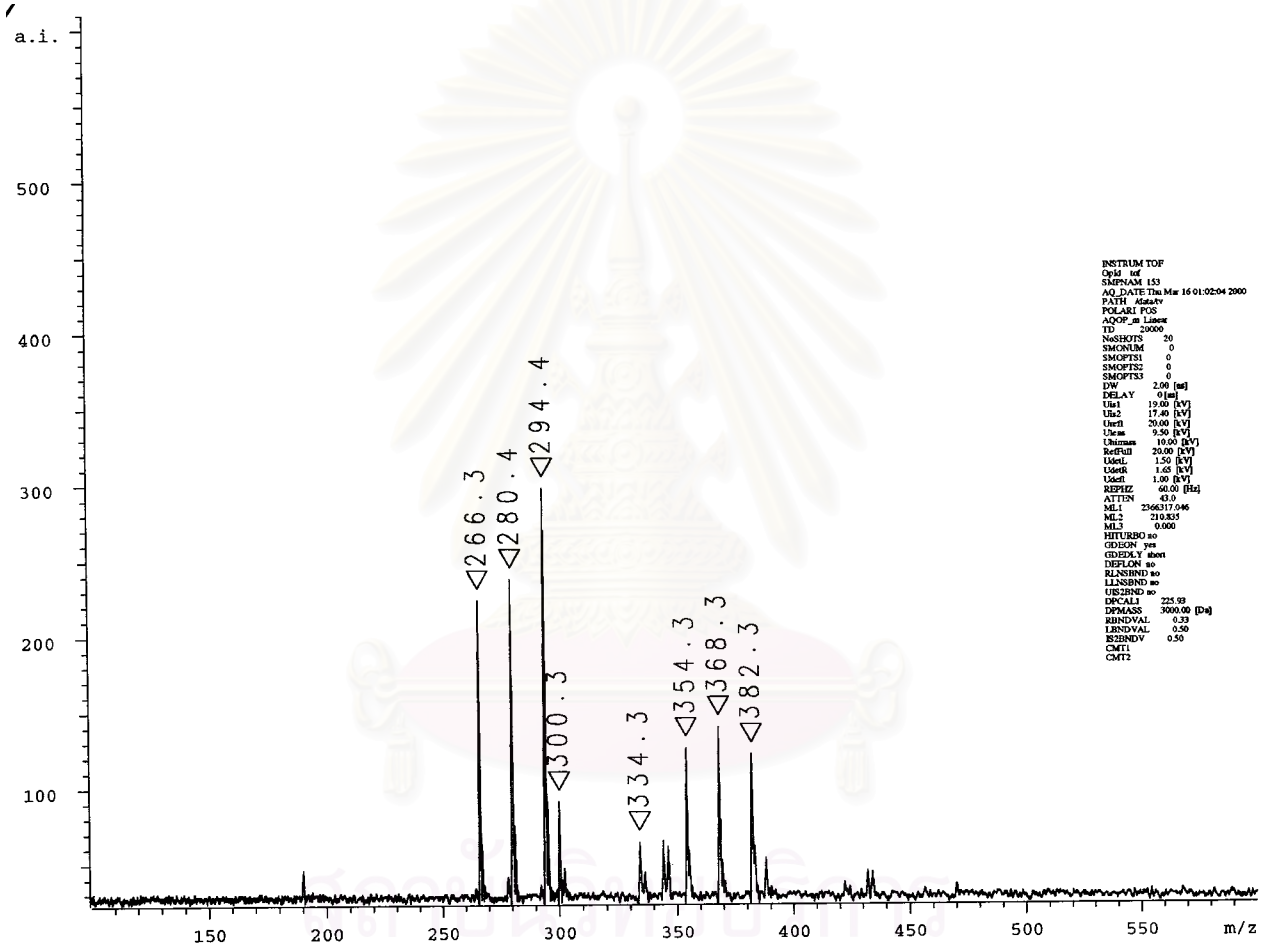

Figure 34: MALDI-TOF mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and benzaldehyde (sub-libraries **11**)

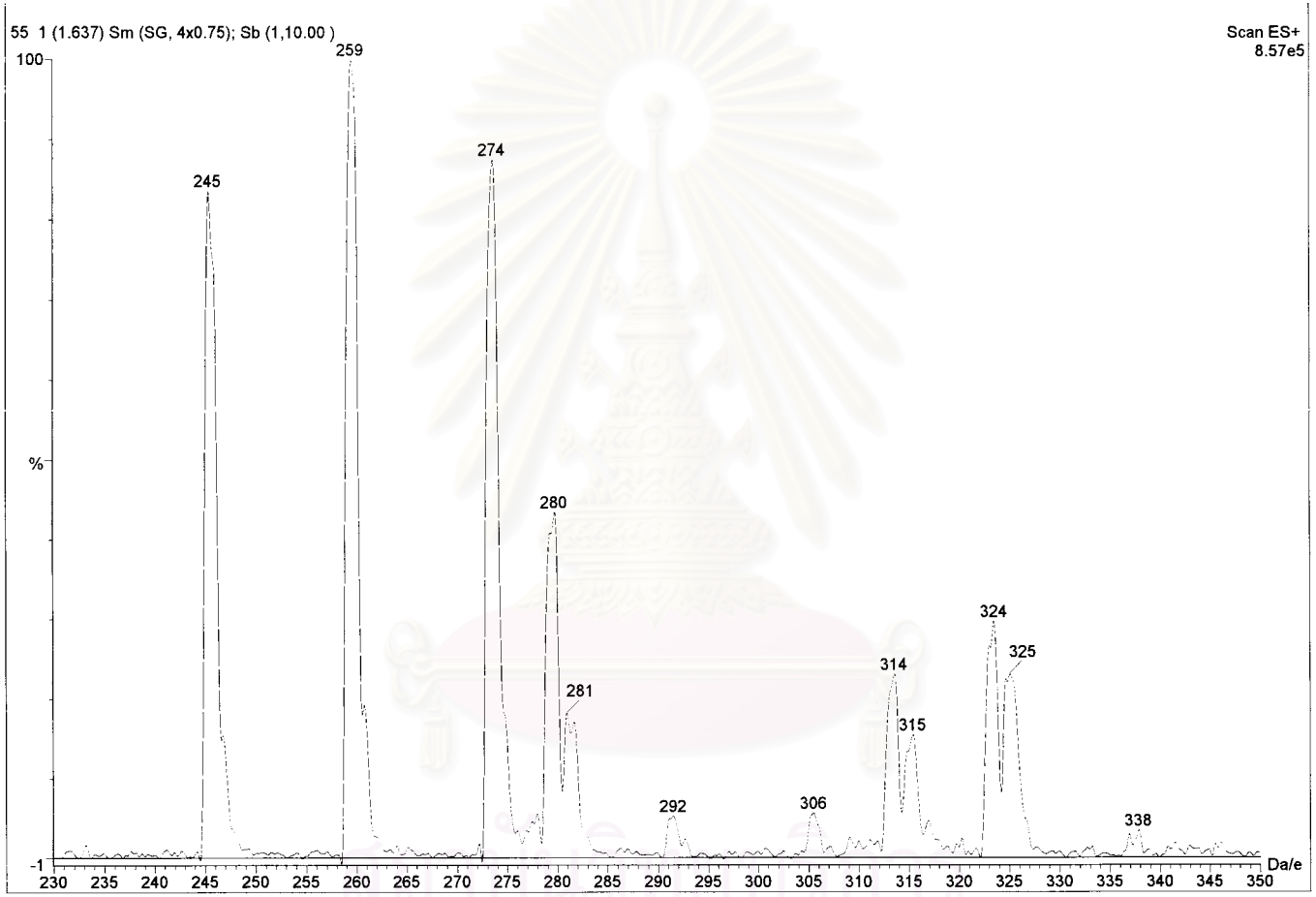

Figure 35: ESI mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and 3-pentanone (sub-libraries **12**)

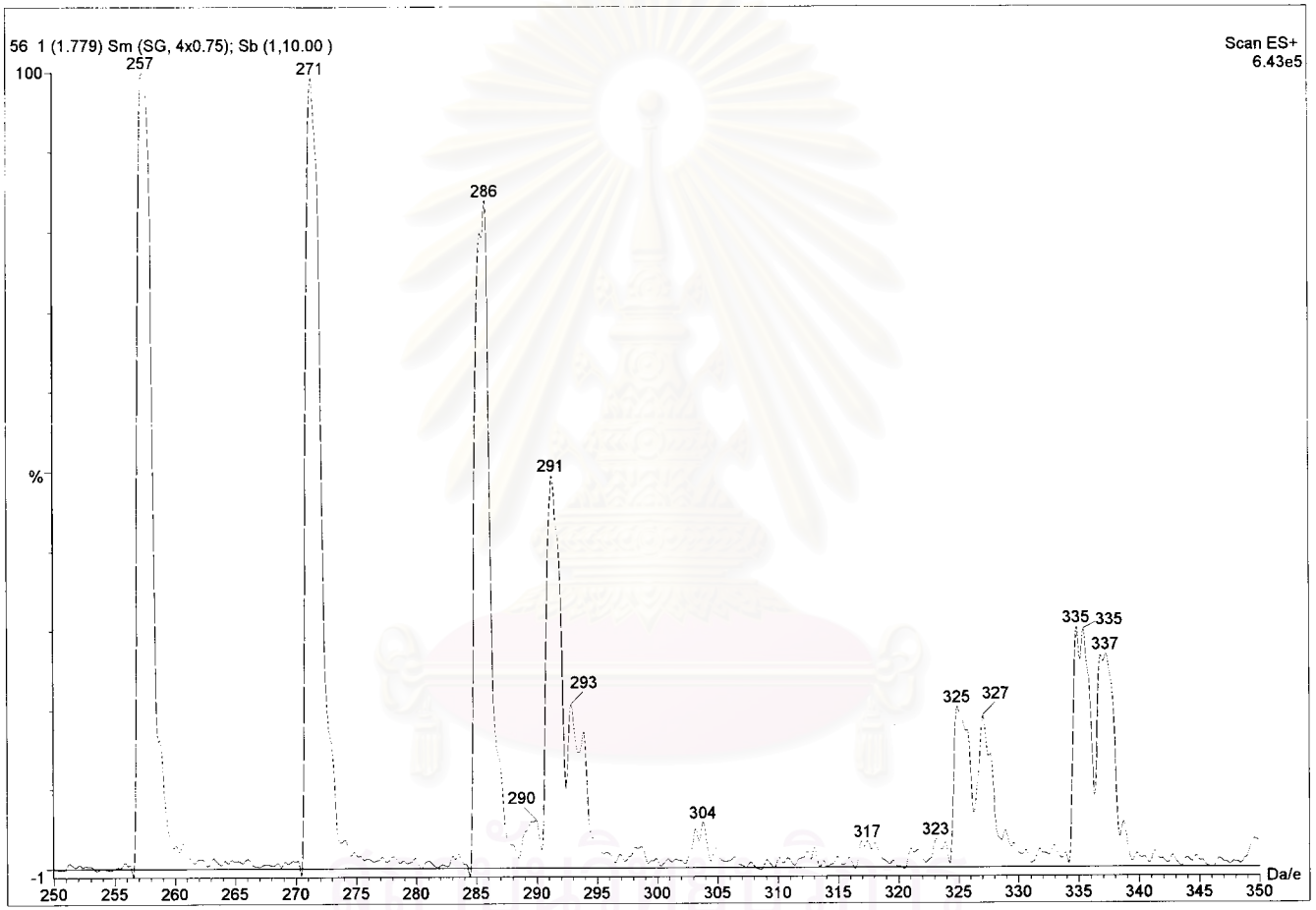

Figure 36: ESI mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and cyclohexanone (sub-libraries **13**)

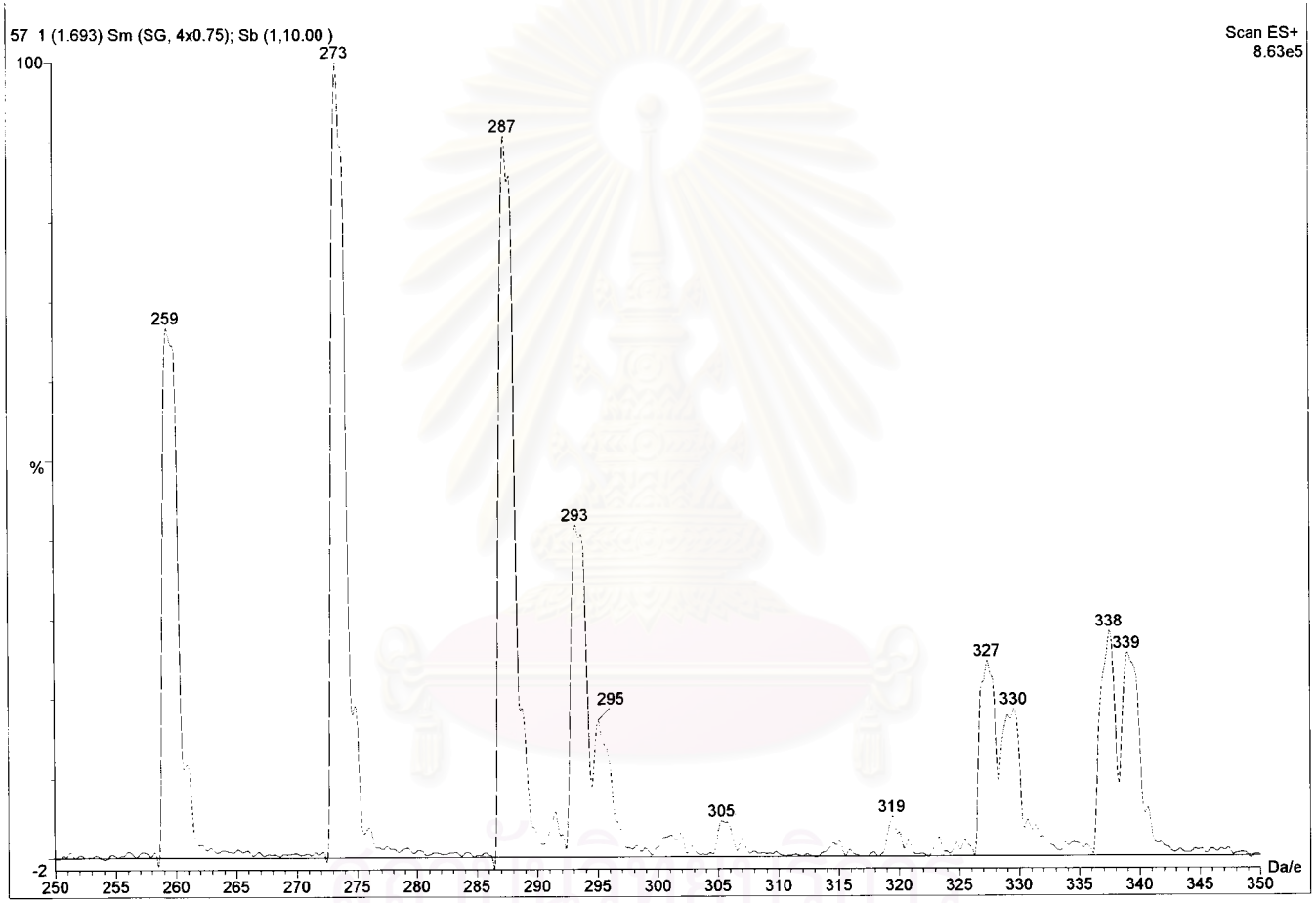

Figure 37: ESI mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and 2-hexanone (sub-libraries **14**)

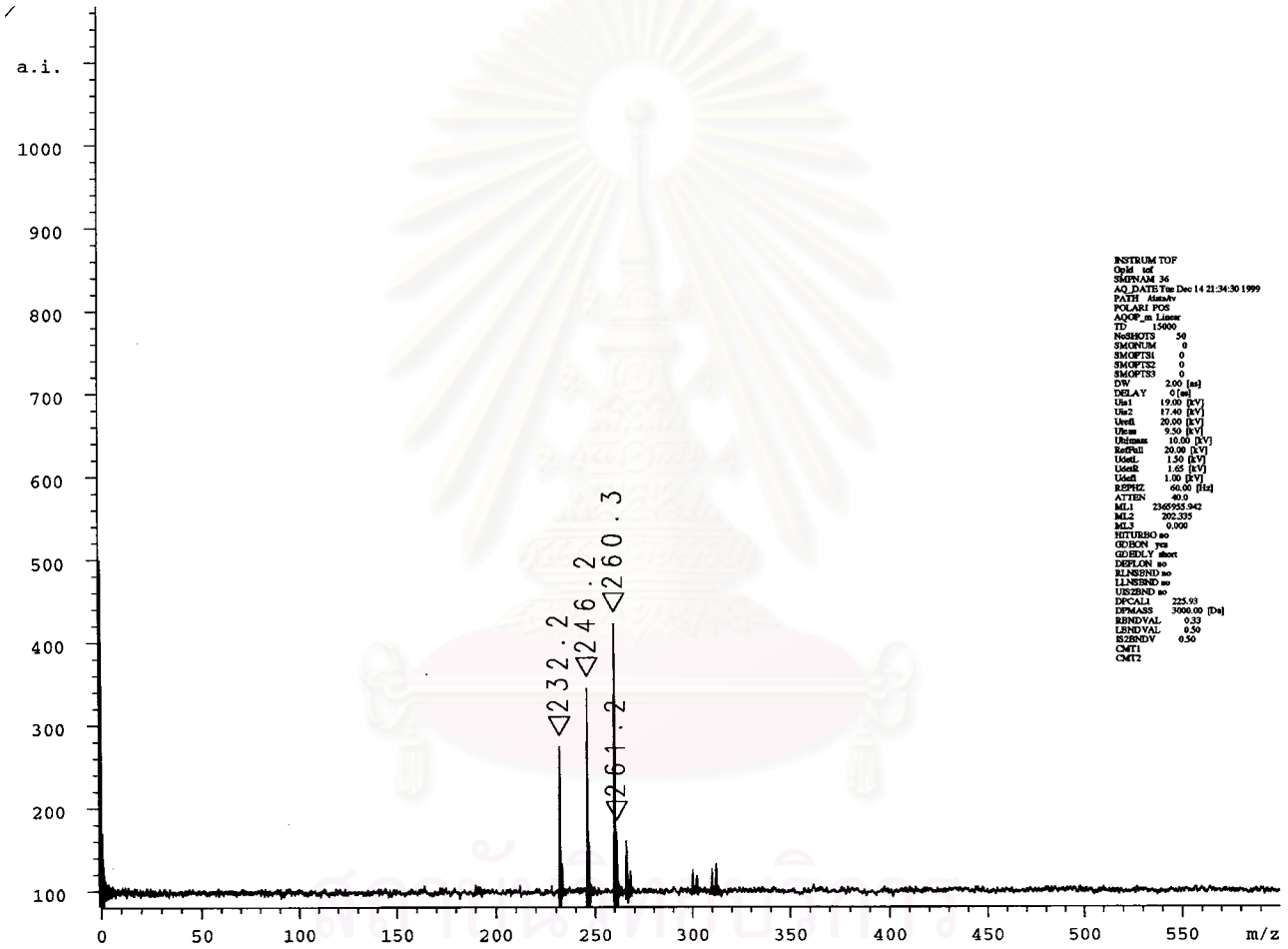

Figure 38: MALDI-TOF mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and 2-butanone (sub-libraries **15**)

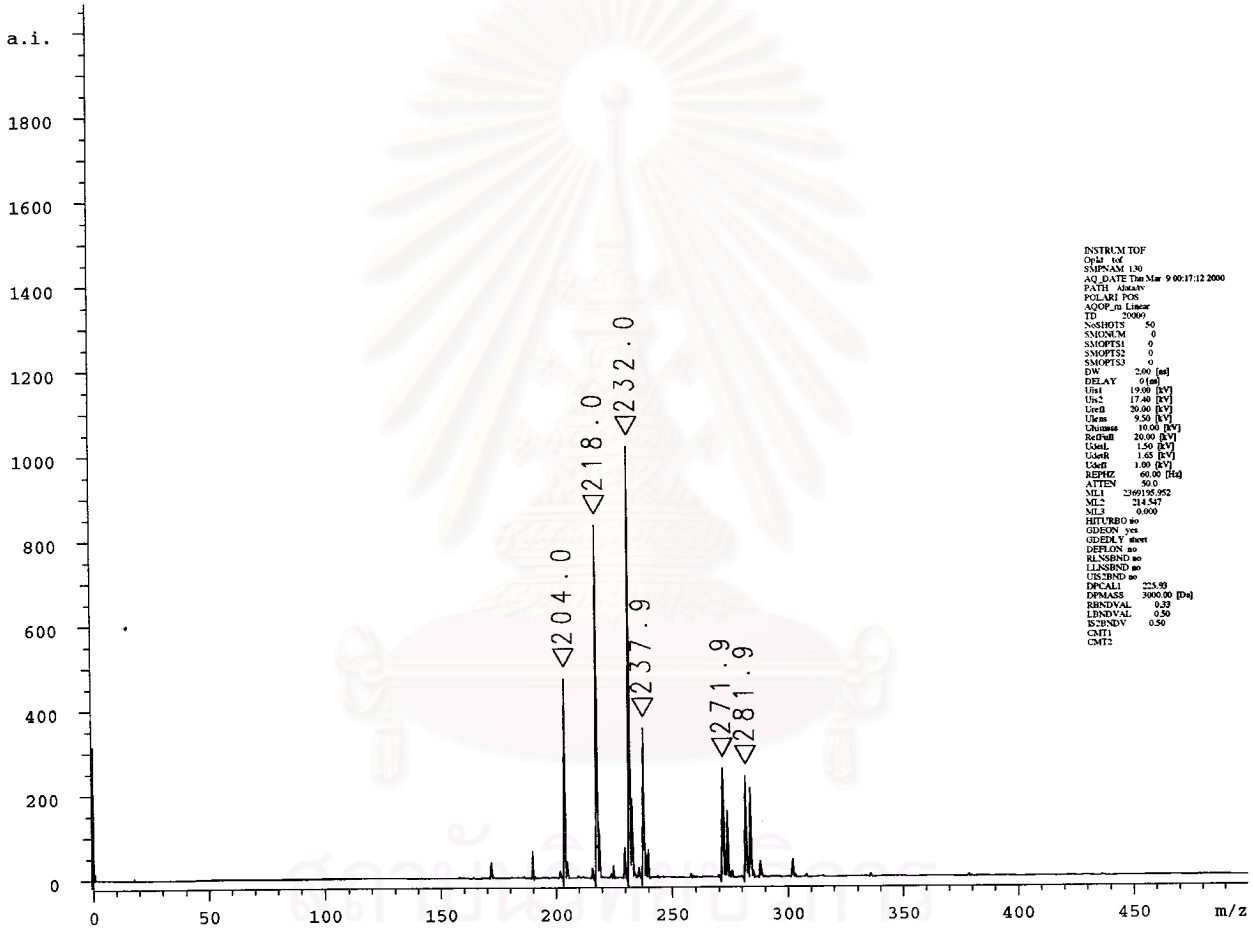

Figure 39: MALDI-TOF mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and acetaldehyde (sub-libraries **16**)

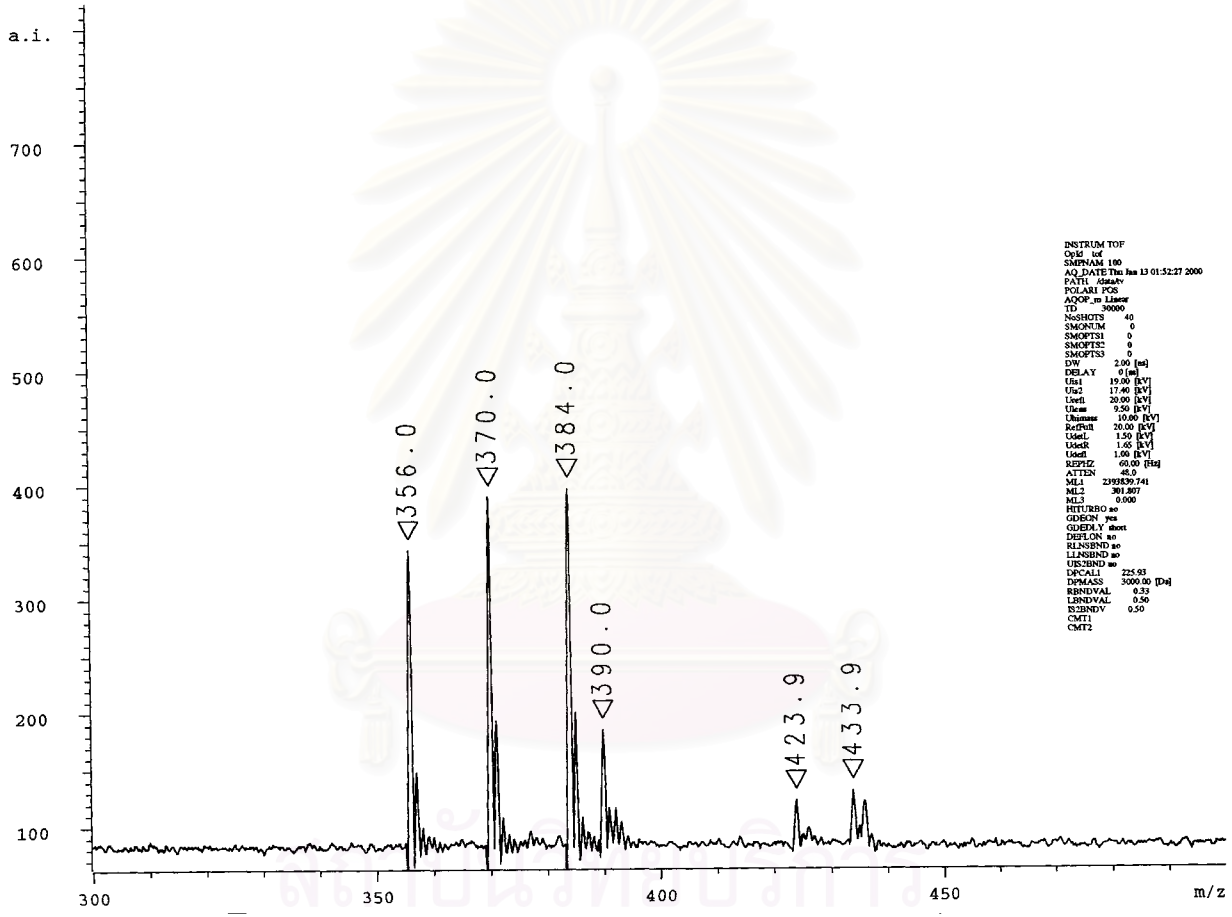

Figure 40: MALDI-TOF mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and 3-phenoxybenzaldehyde (sub-libraries **17**)

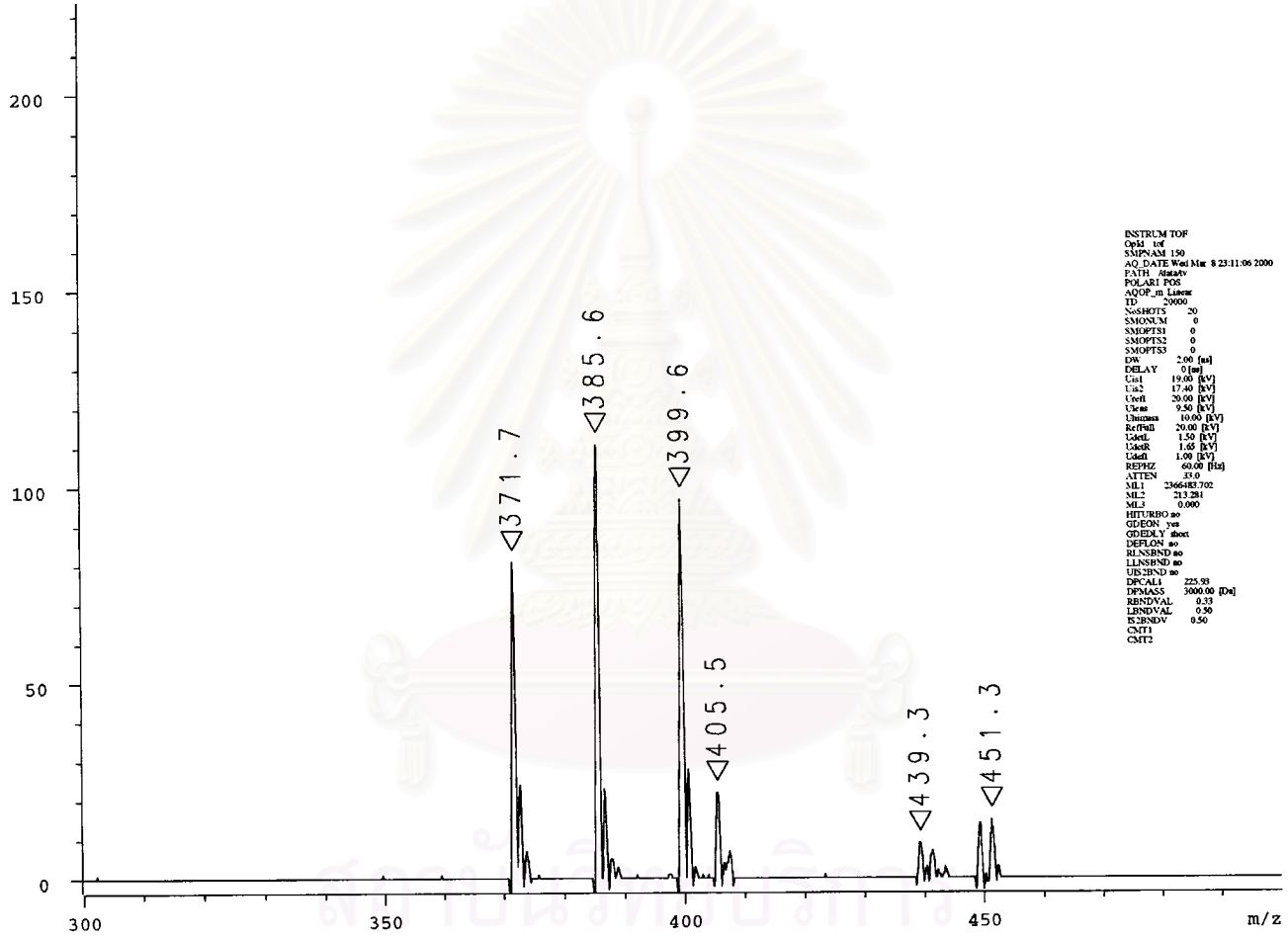

Figure 41: MALDI-TOF mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and 4-benzyloxybenzaldehyde (sub-libraries **18**)

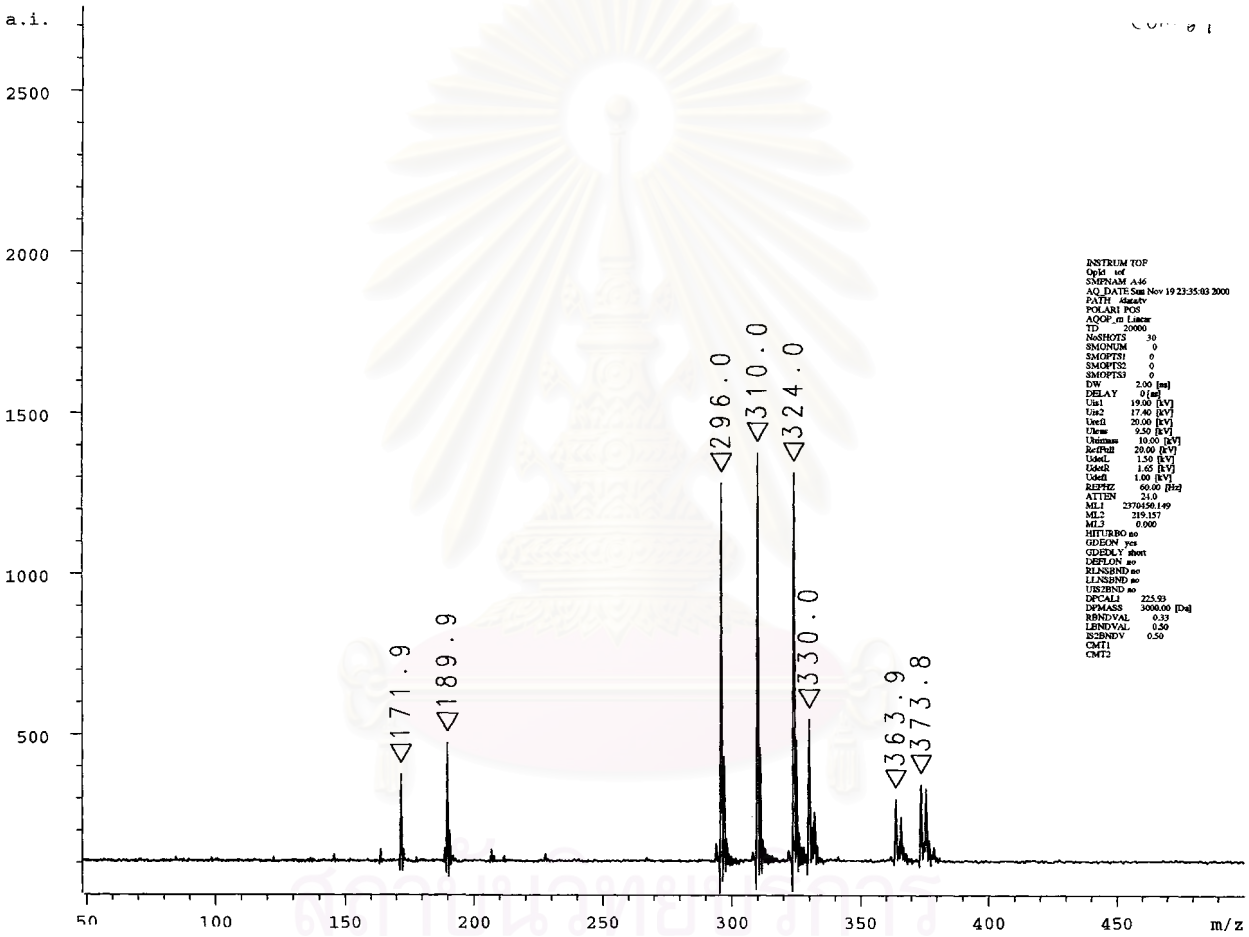

Figure 42: MALDI-TOF mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and 4-methoxybenzaldehyde (sub-libraries **19**)

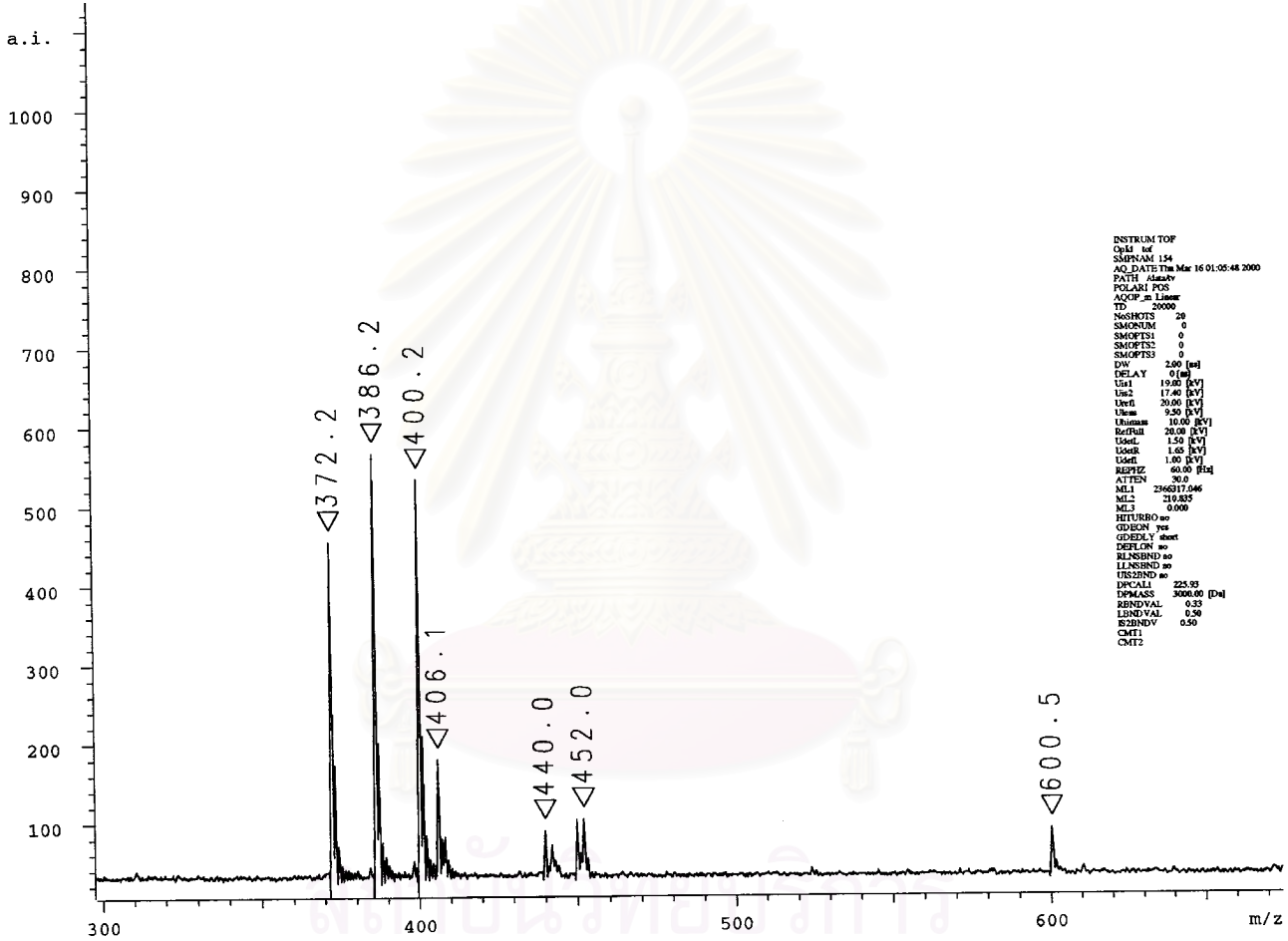

Figure 43: MALDI-TOF mass spectrum of reaction between a mixture of arylbiguanide hydrochloride and 3-benzyloxybenzaldehyde (sub-libraries **20**)

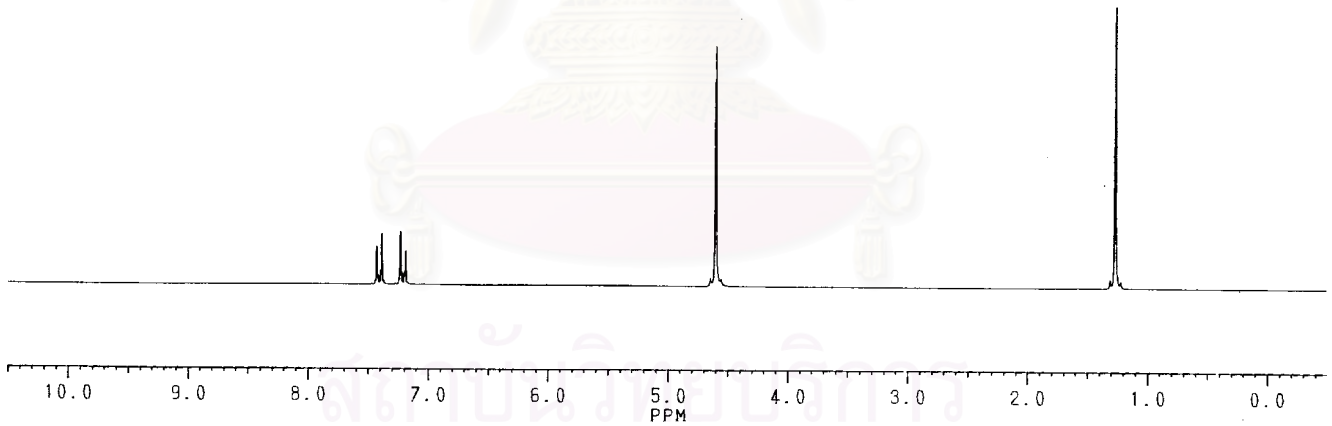

Figure 44: ^{1}H NMR spectrum (DMSO, 200 MHz) of 1-(4′-chlorophenyl)-2,2-dimethyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-1)**

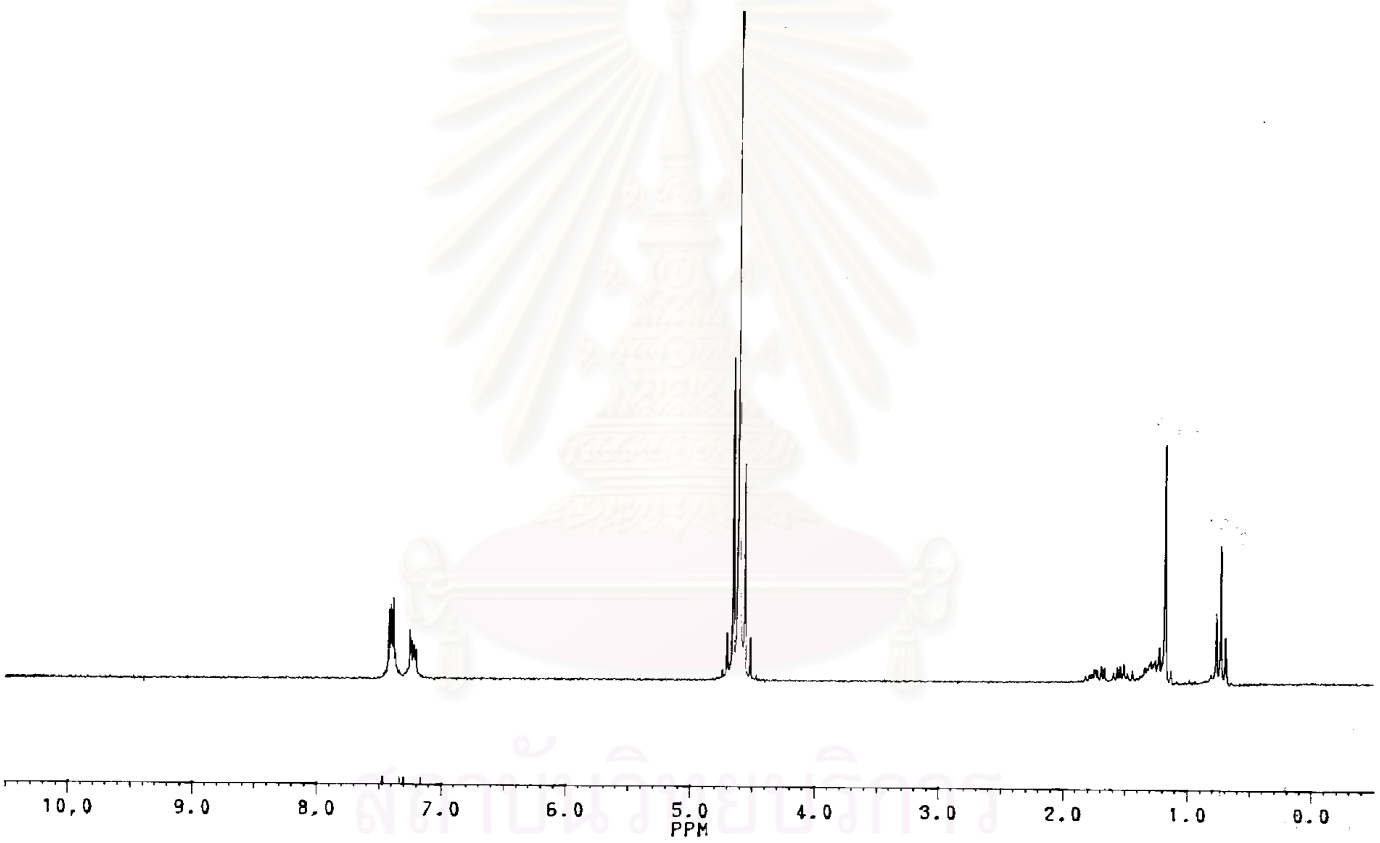

Figure 45: ^{1}H NMR spectrum (D_2O, 200 MHz) of 1-phenyl-2-methyl-2-propyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-2)**

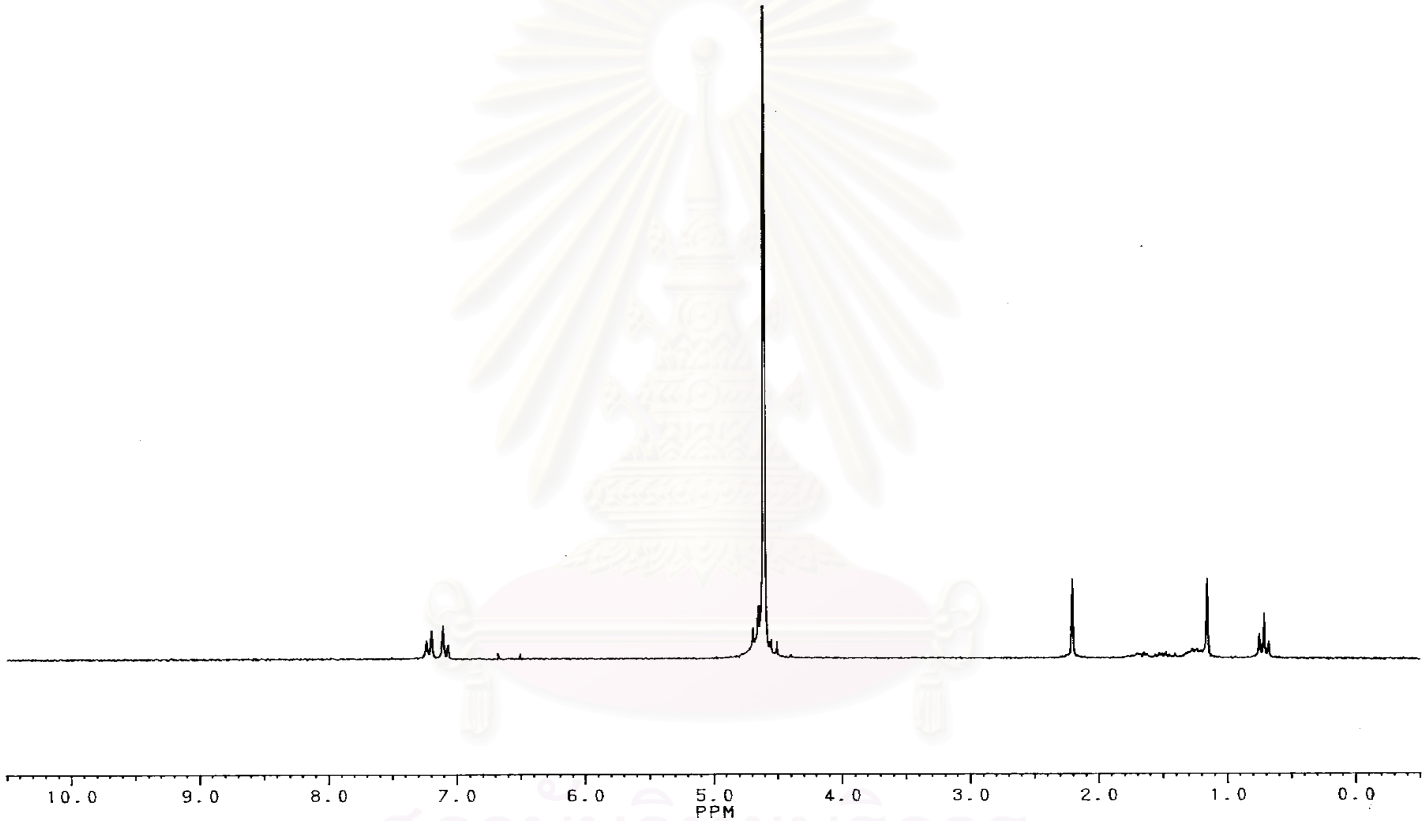

Figure 46: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(4′-methylphenyl)-2-methyl-2-propyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-3)**

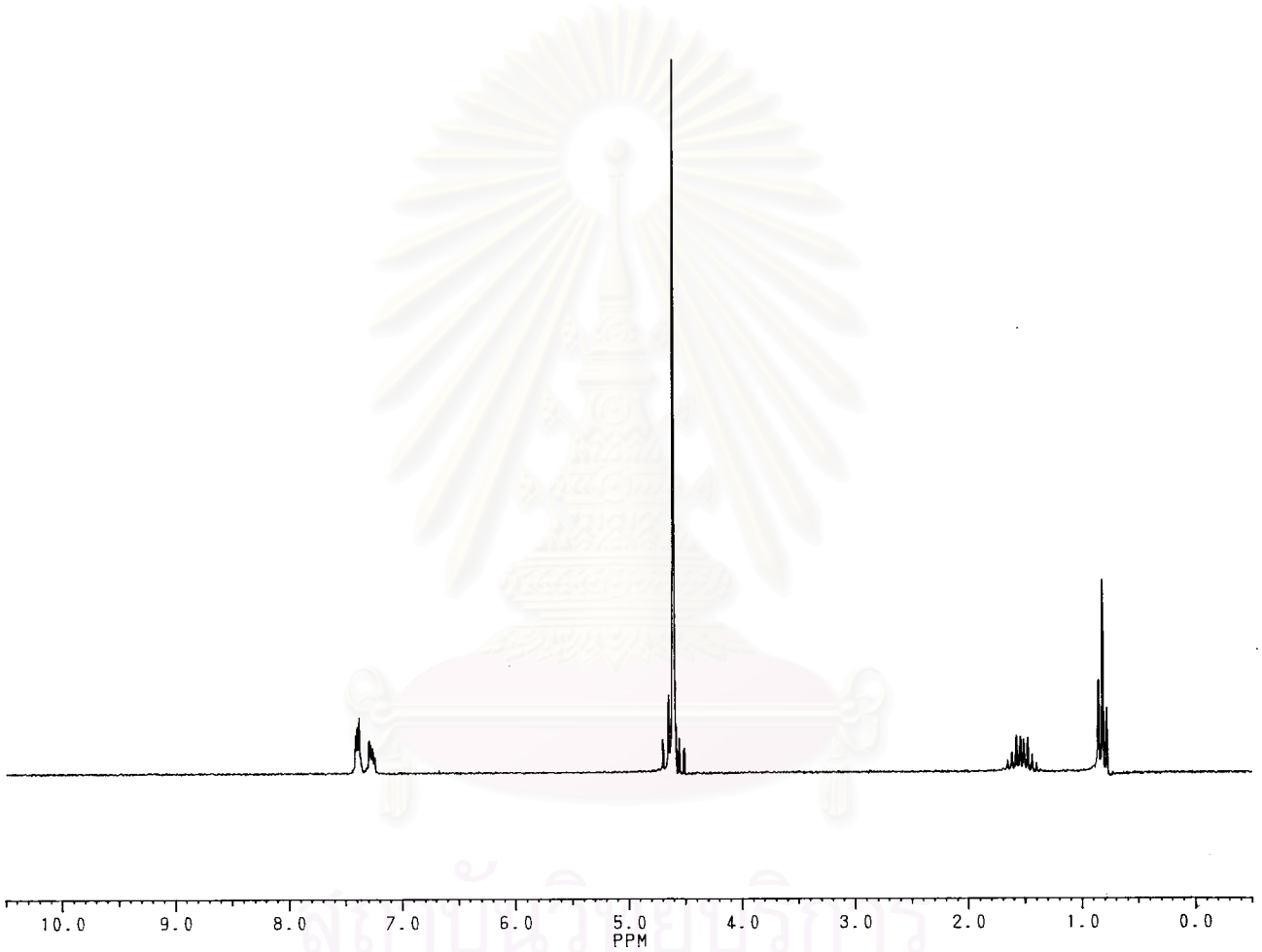

Figure 47: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-phenyl-2,2-diethyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-4)**

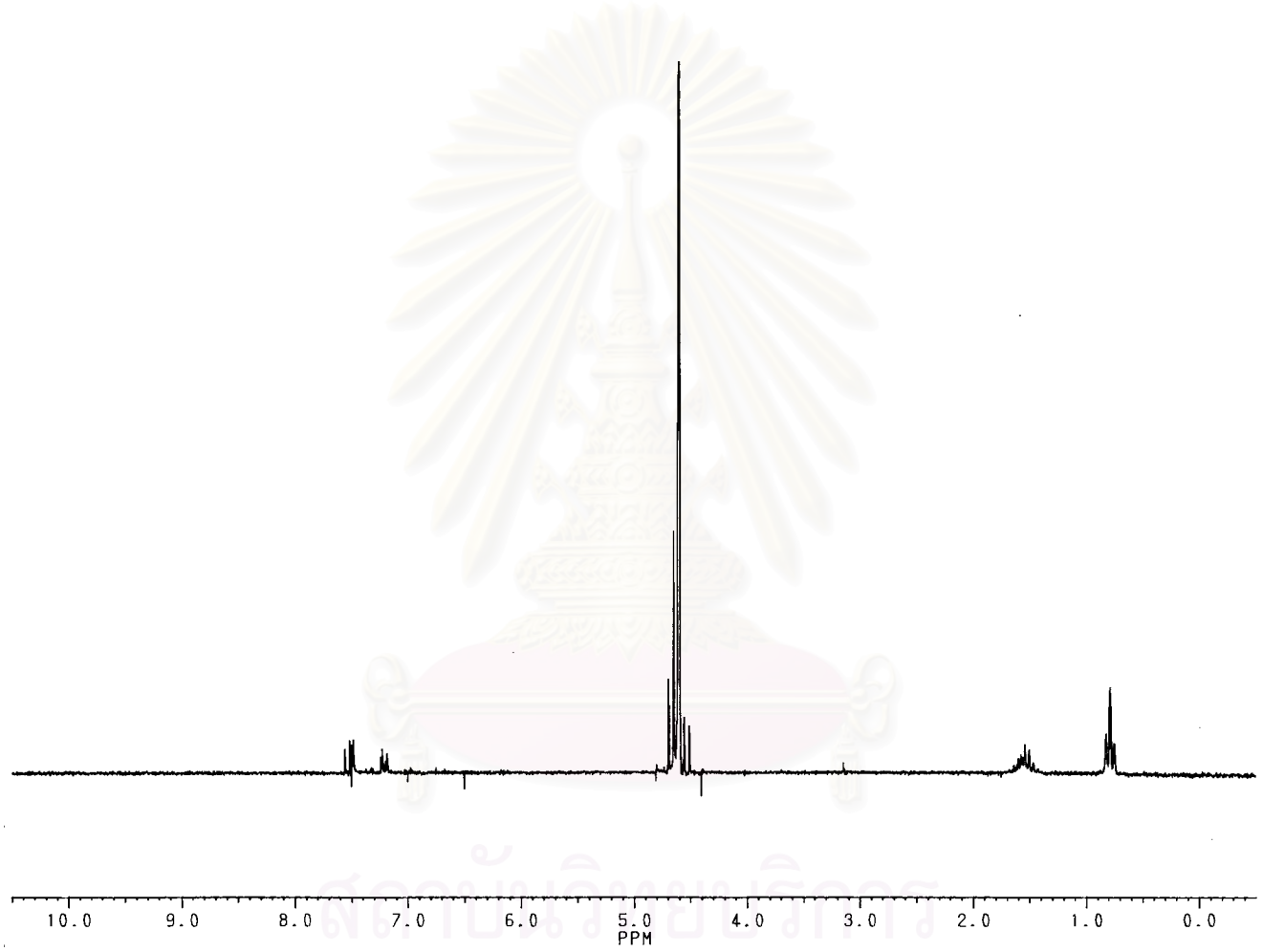

Figure 48: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(3′,4′-dichlorophenyl)-2,2-diethyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-5)**

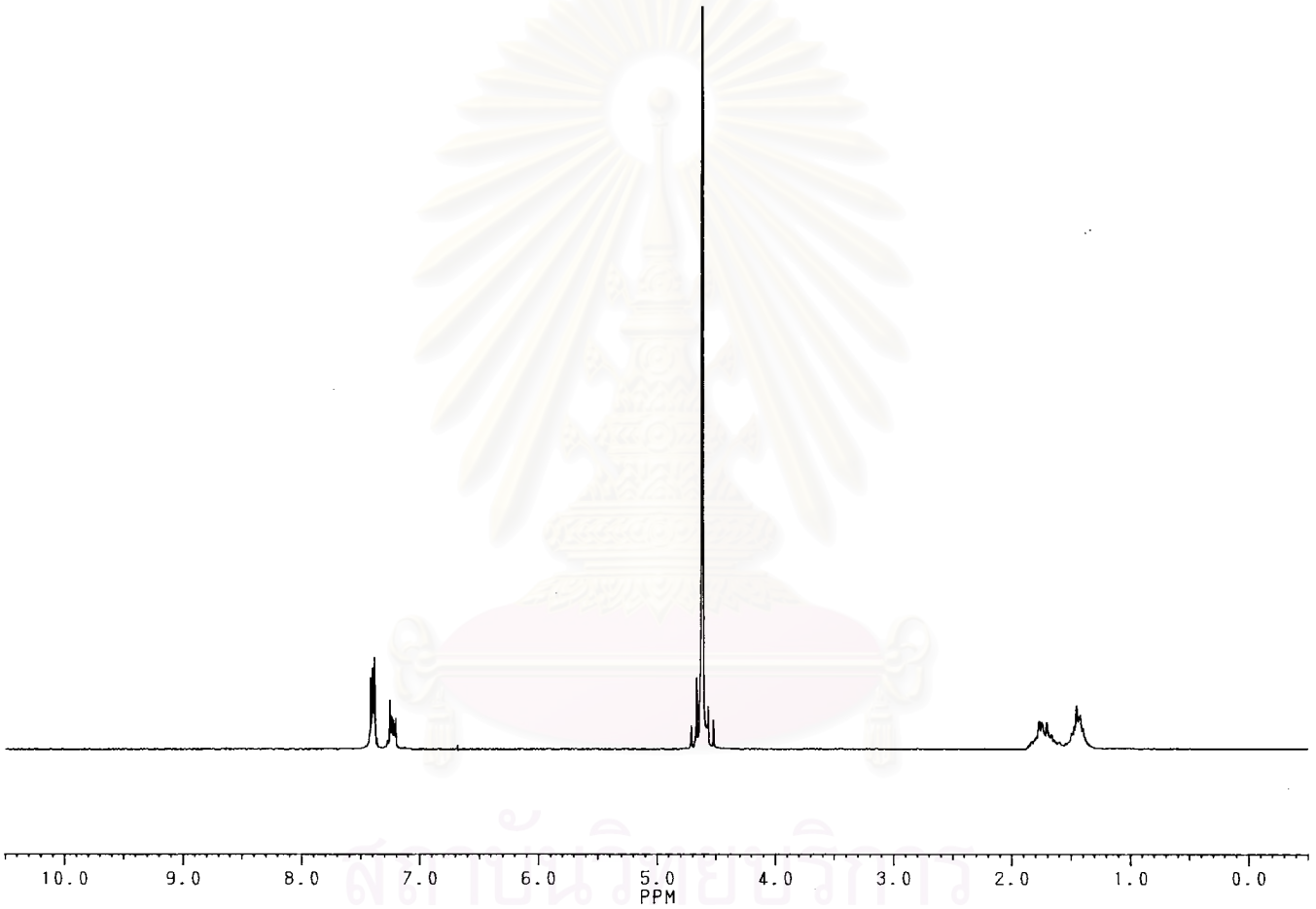

Figure 49: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-phenyl-2,2-cyclopentylidene-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-6)**

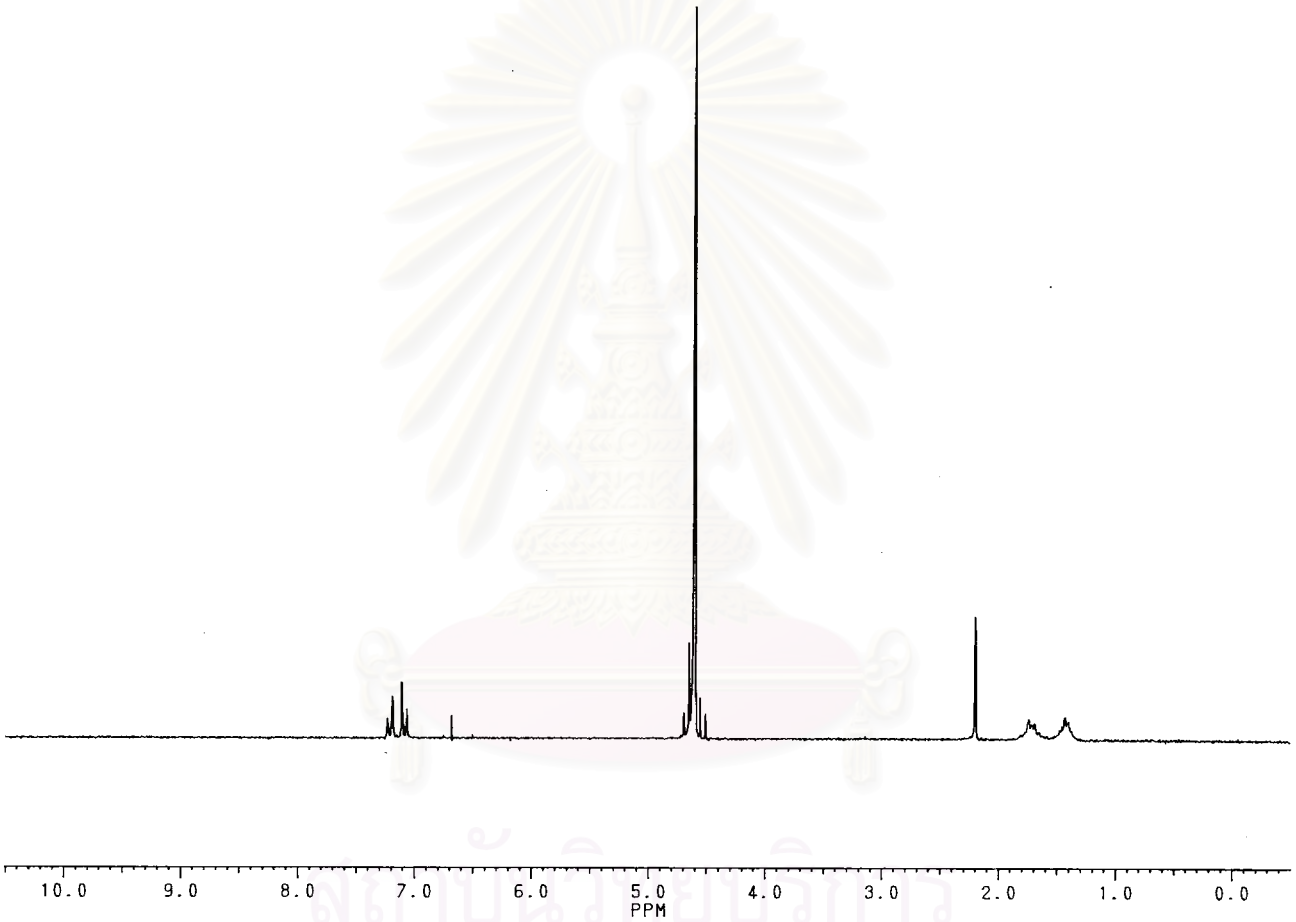

Figure 50: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(4′-methylphenyl)-2,2-cyclopentylidene-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-7)**

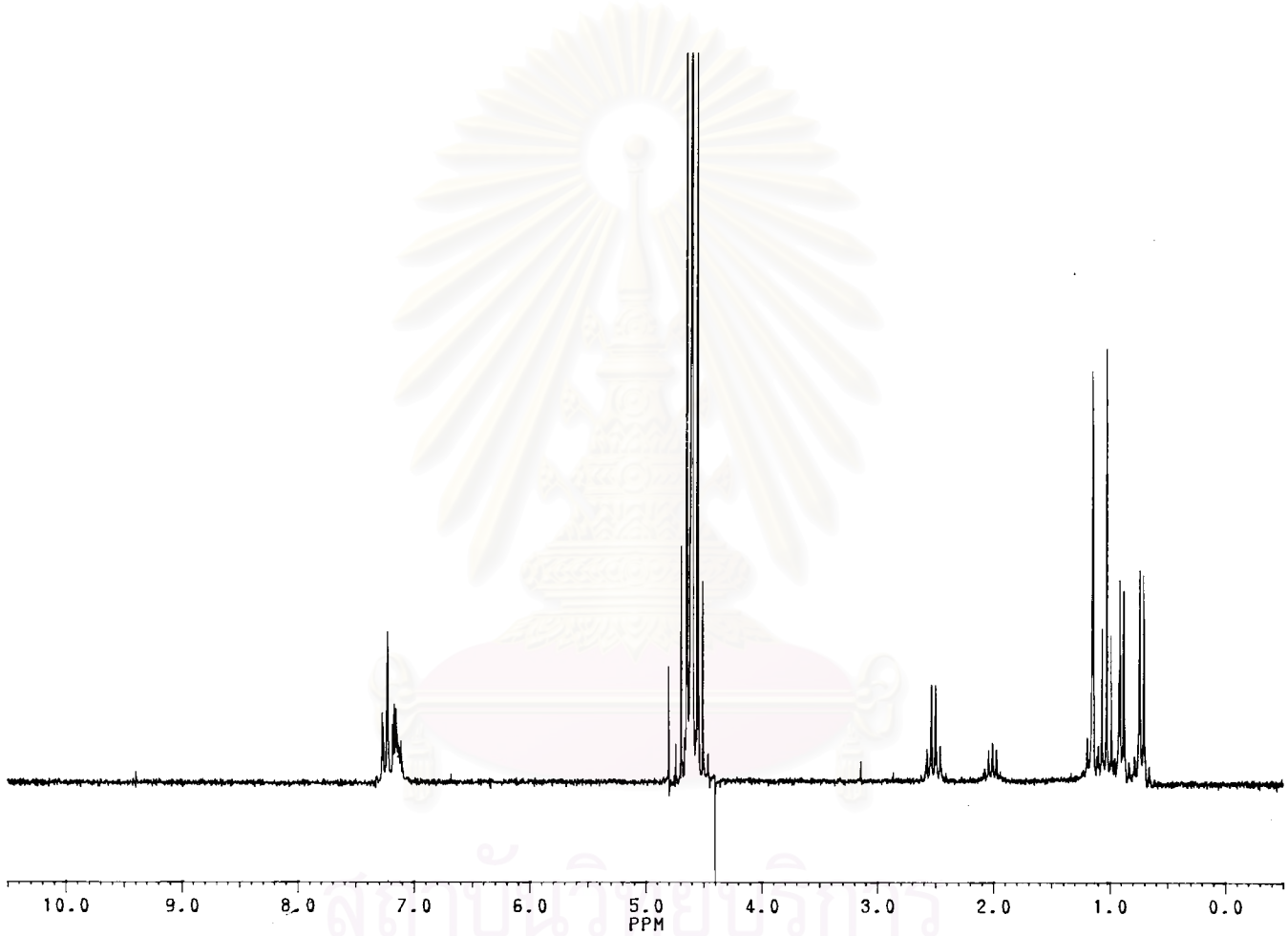

Figure 51: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(4′-ethylphenyl)-2-isopropyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-8)**

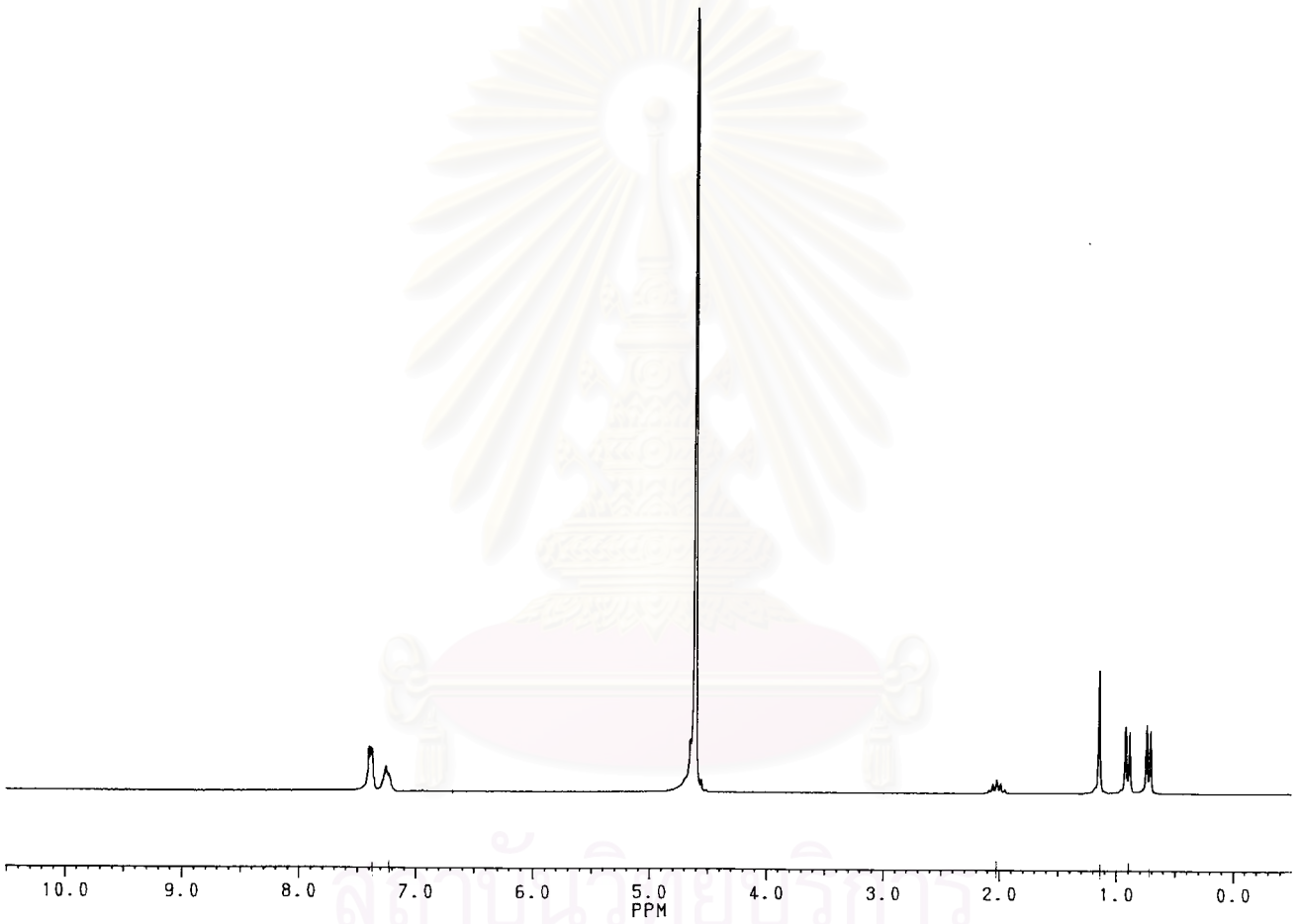

Figure 52: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-phenyl-2-isopropyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-9)**

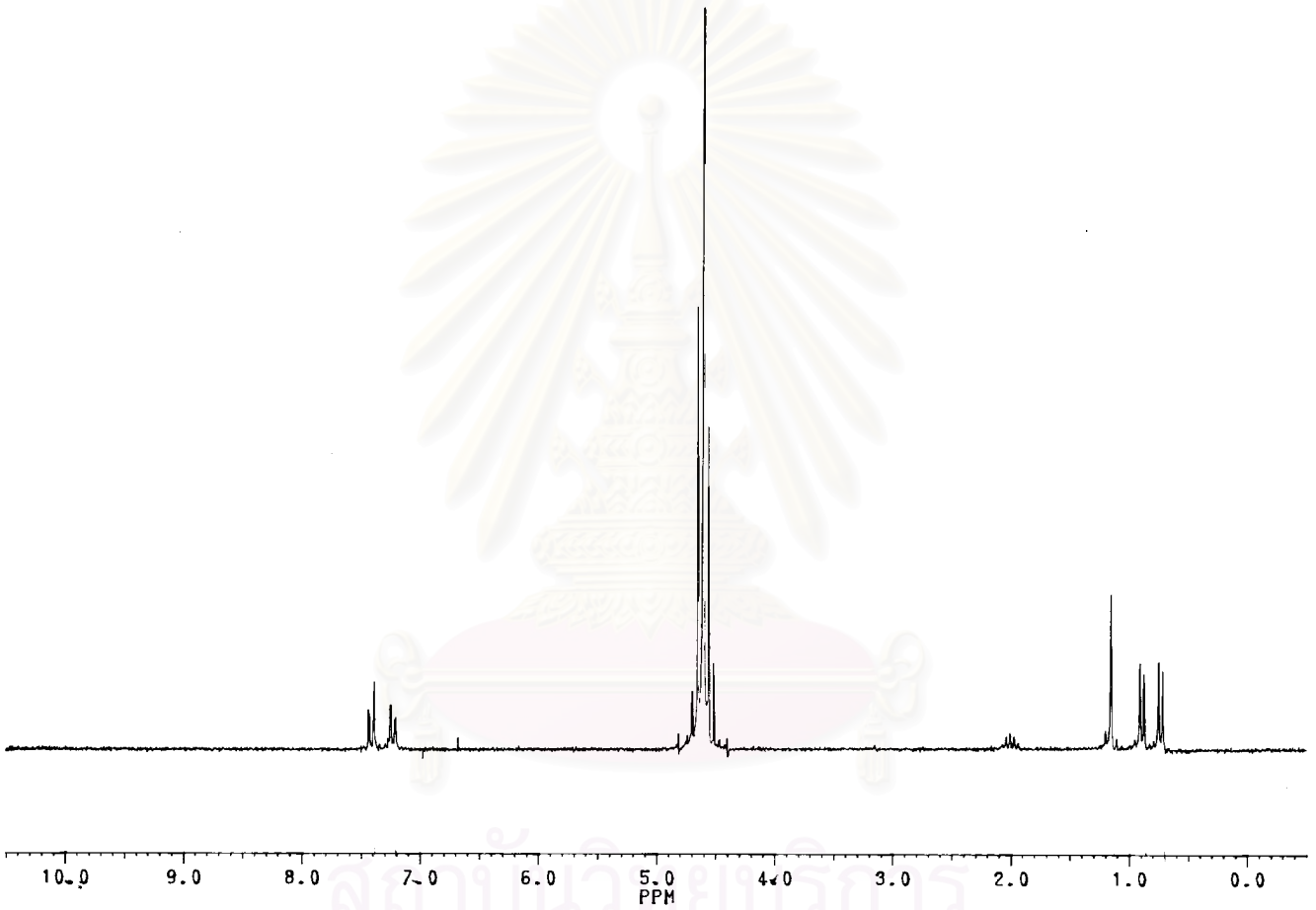

Figure 53: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(4′-chlorophenyl)-2-isopropyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-10)**

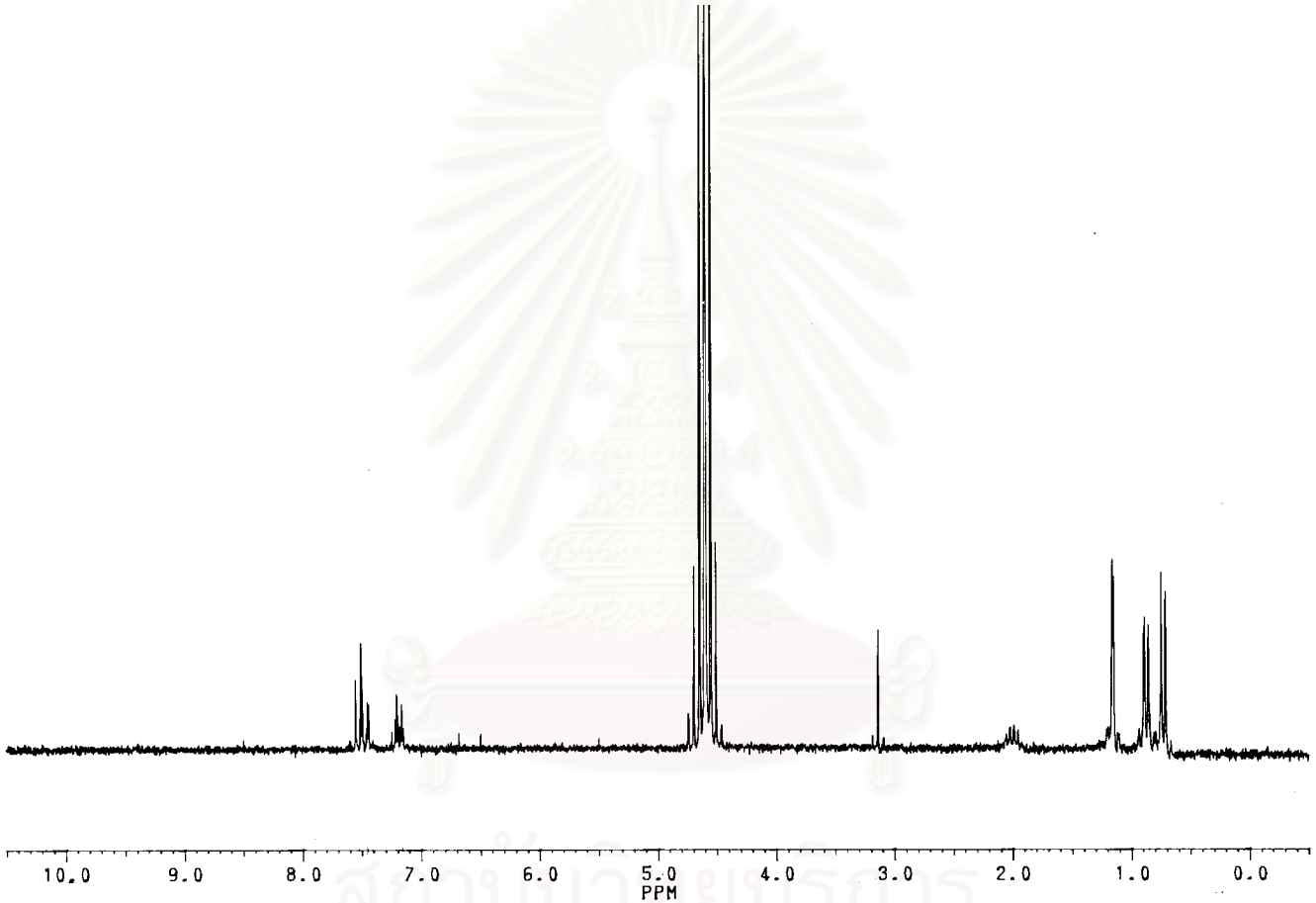

Figure 54: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(3′,4′-dichlorophenyl)-2-isopropyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-11)**

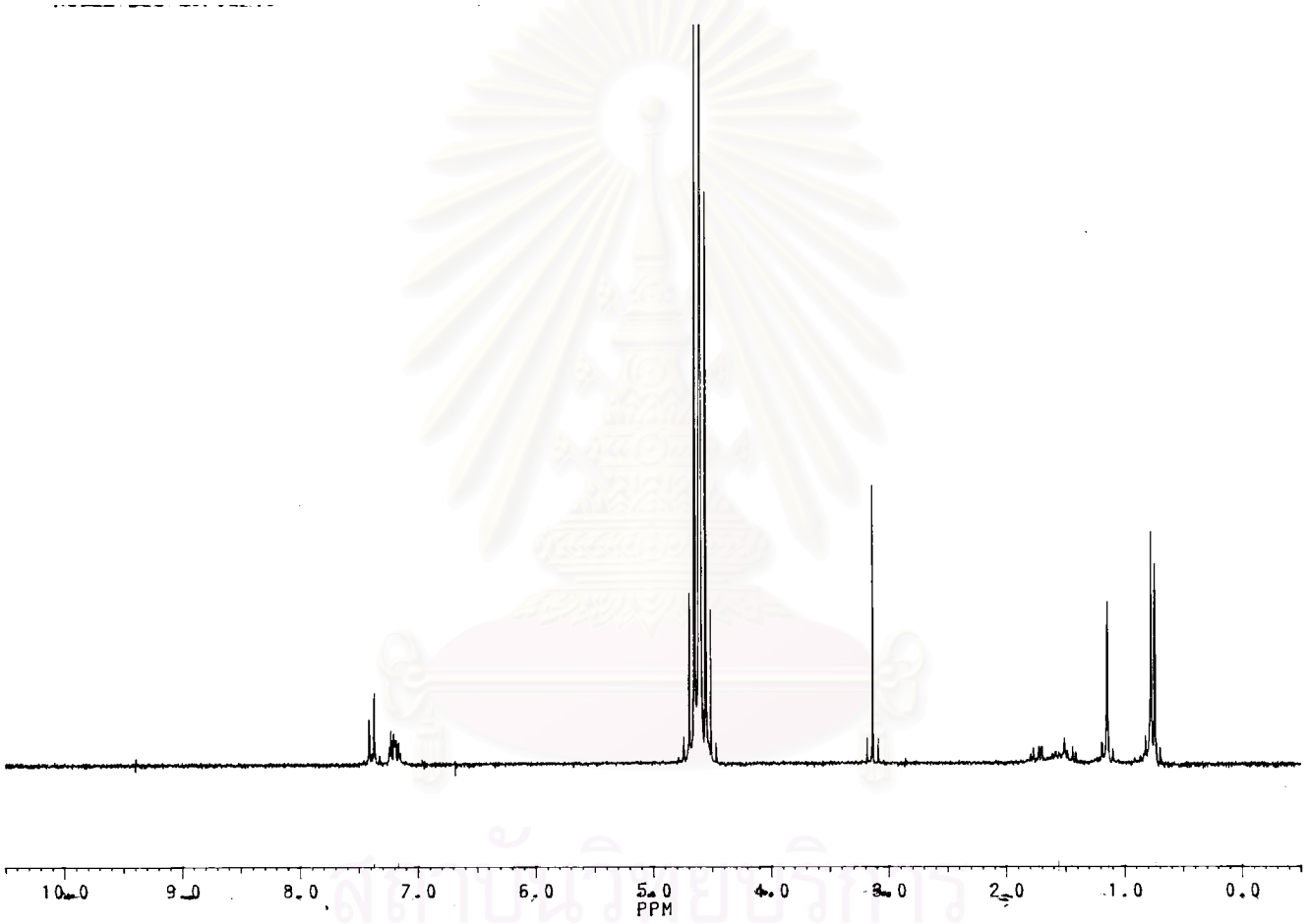

Figure 55: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(4′-methylphenyl)-2-isobutyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride. H_2O **(2-12)**

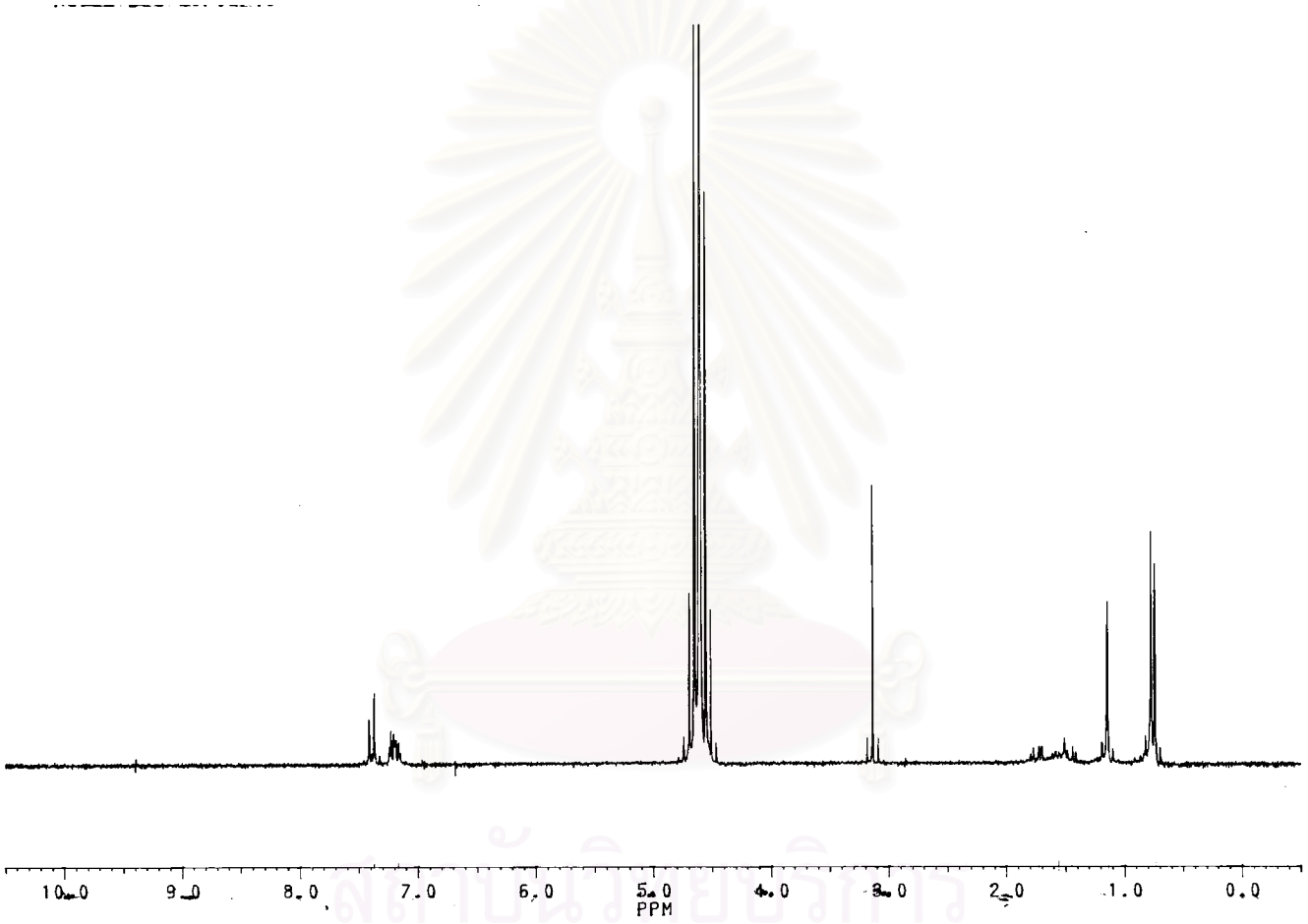

Figure 56: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(4′-chlorophenyl)-2-isobutyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride.H_2O **(2-13)**

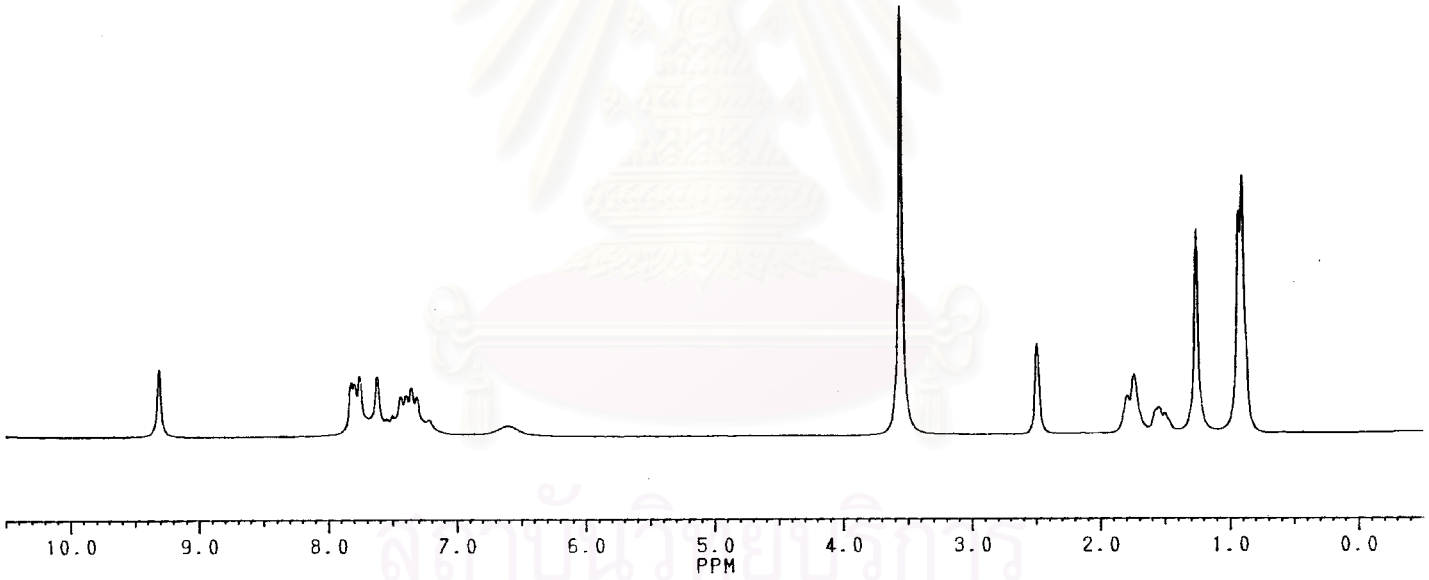

Figure 57: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(3′,4′-dichlorophenyl)-2-isobutyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-14)**

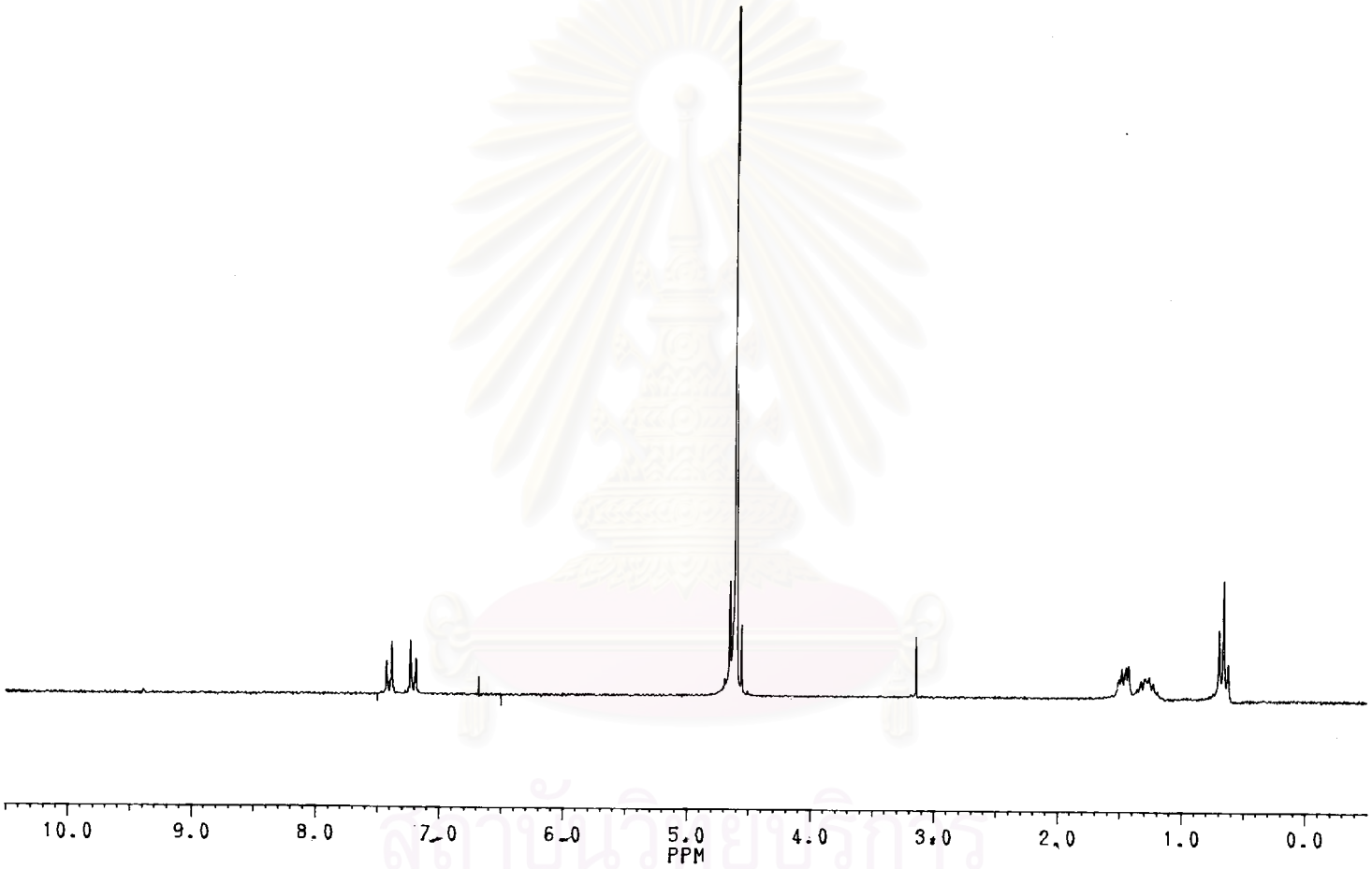

Figure 58: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(3′,4′-dichlorophenyl)-2-isopentyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-15)**

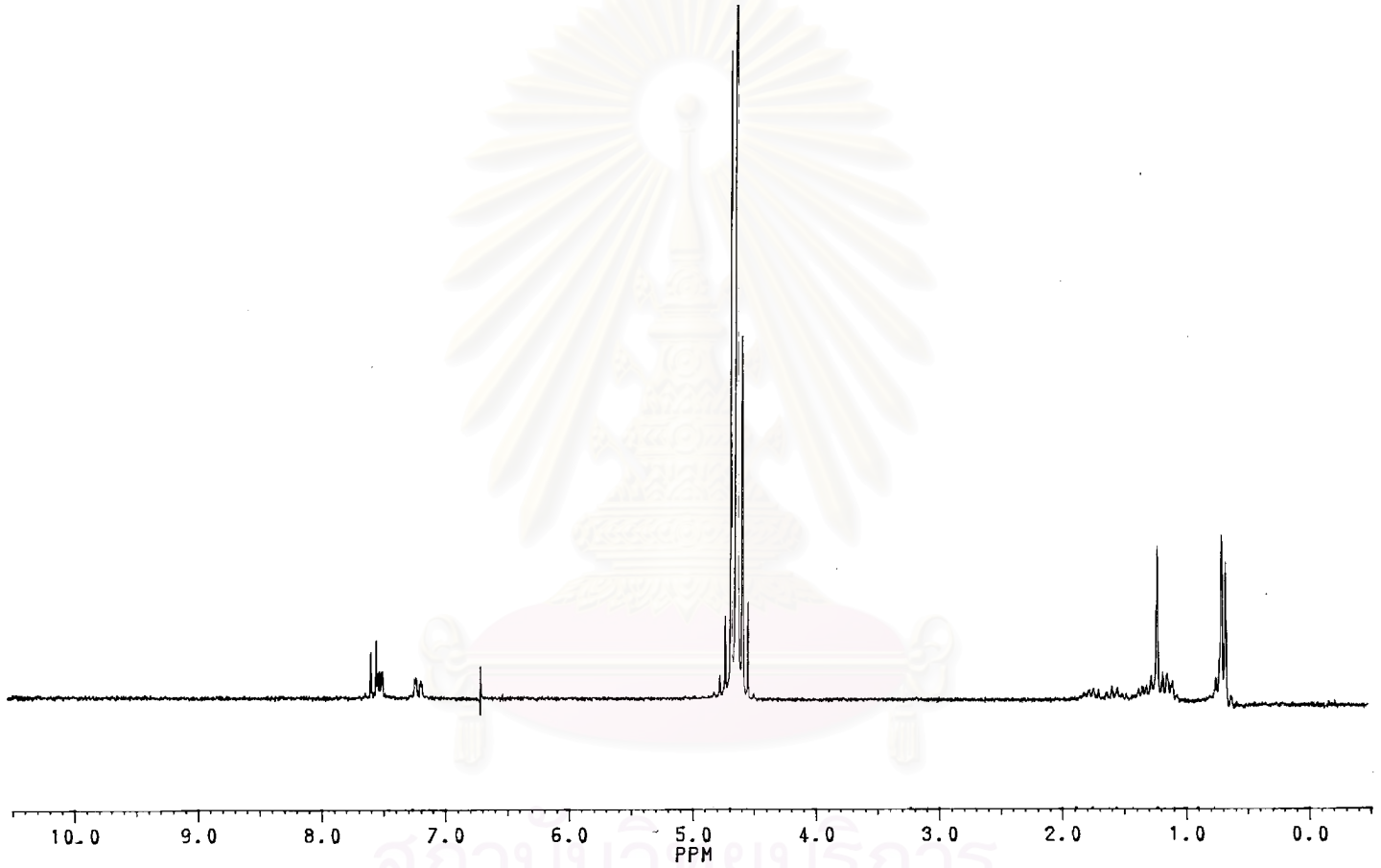

Figure 59: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(4′-chlorophenyl)-2,2-dipropyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-16)**

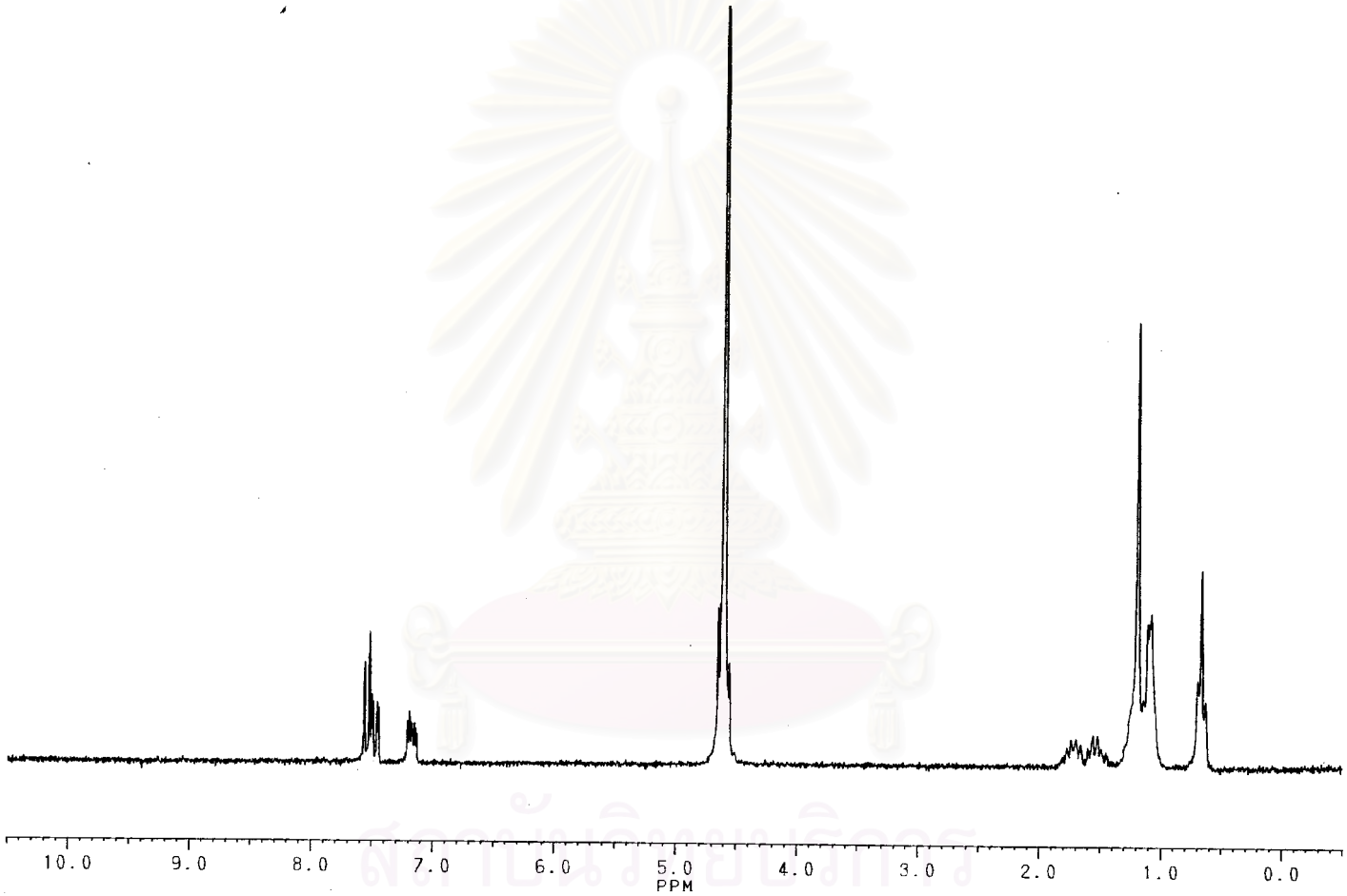

Figure 60: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(3′,4′-dichlorophenyl)-2-methyl-2-pentyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-17)**

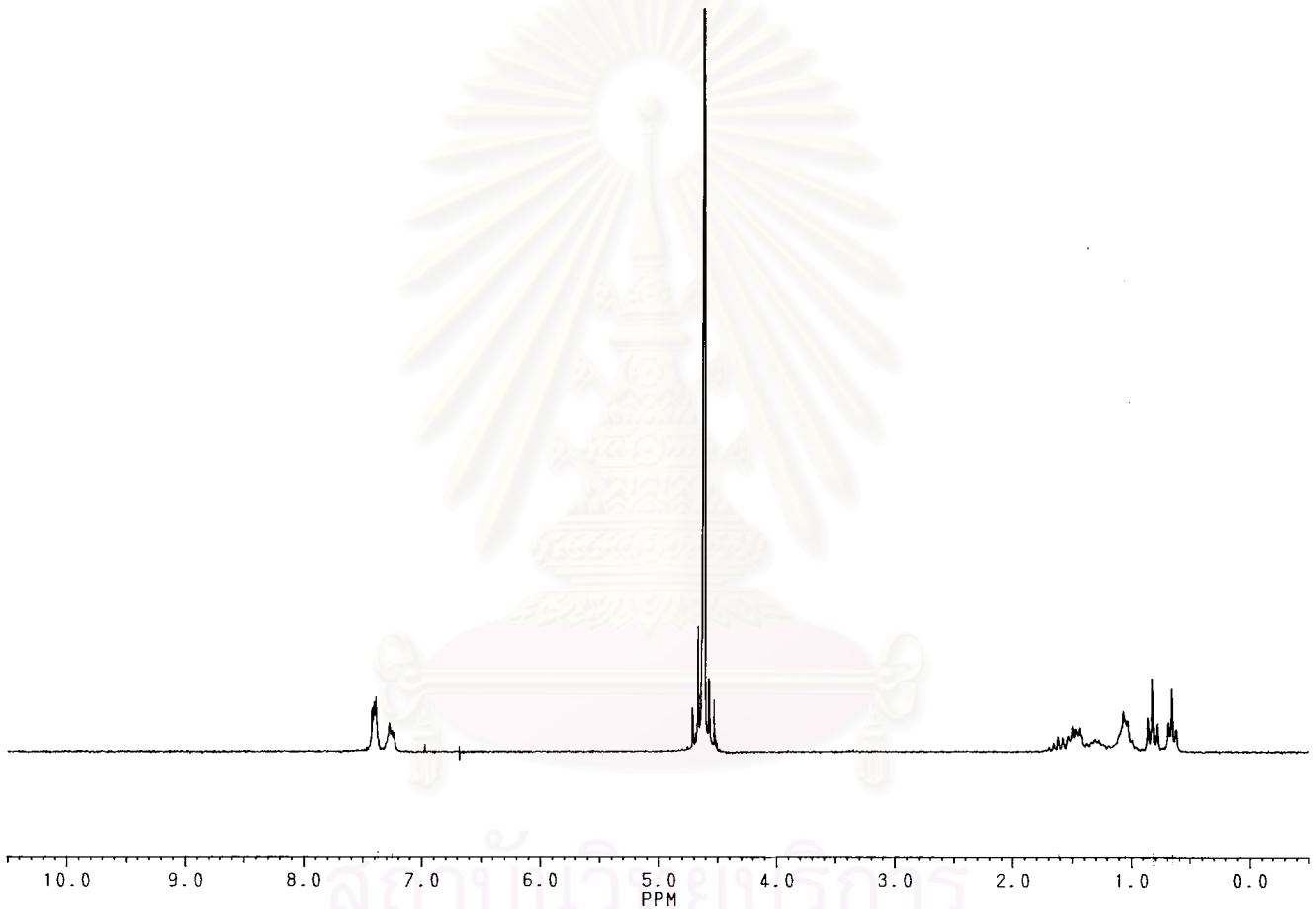

Figure 61: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-phenyl-2-ethyl-2-pentyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-18)**

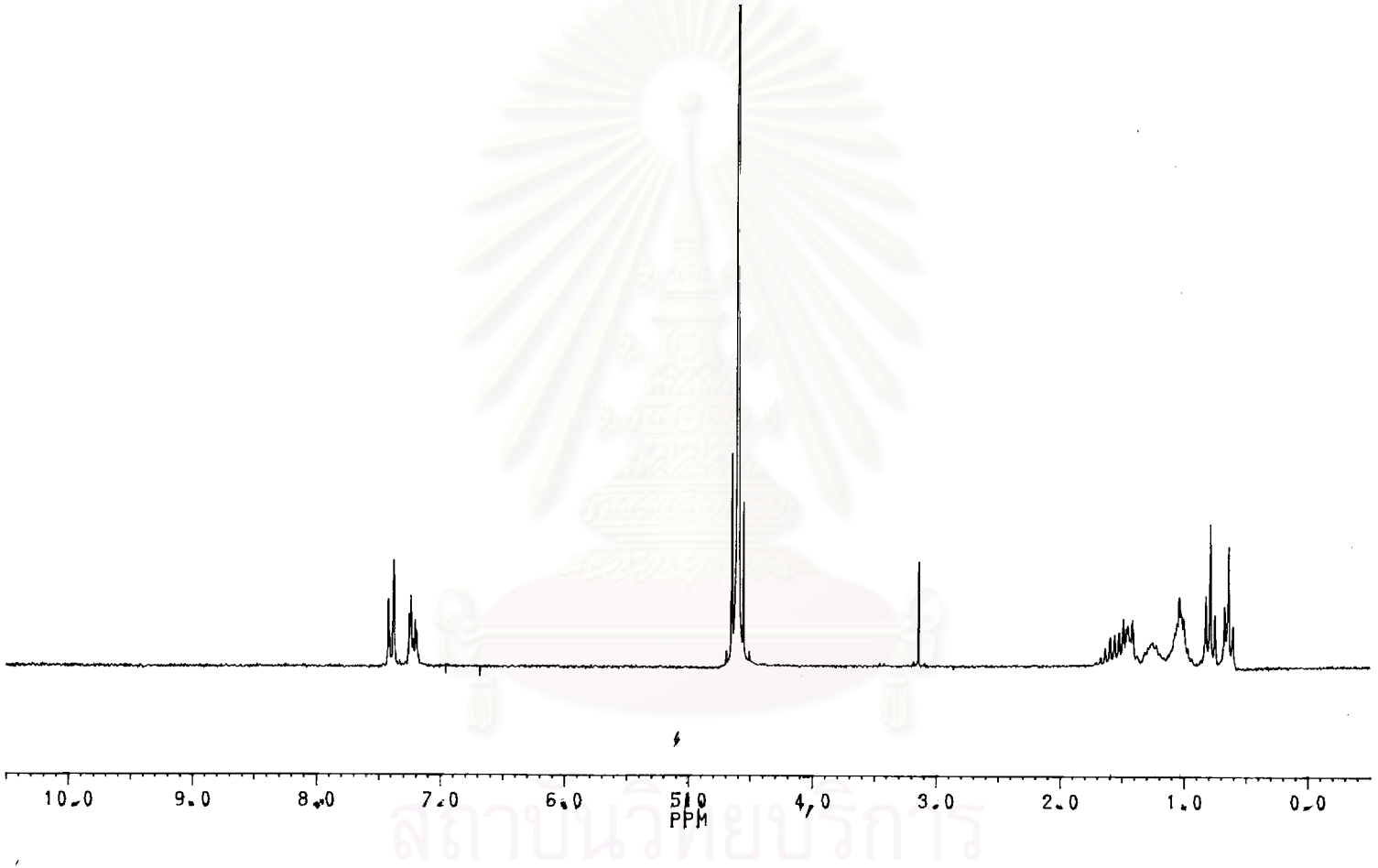

Figure 62: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(4′-chlorophenyl)-2-ethyl-2-pentyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-19)**

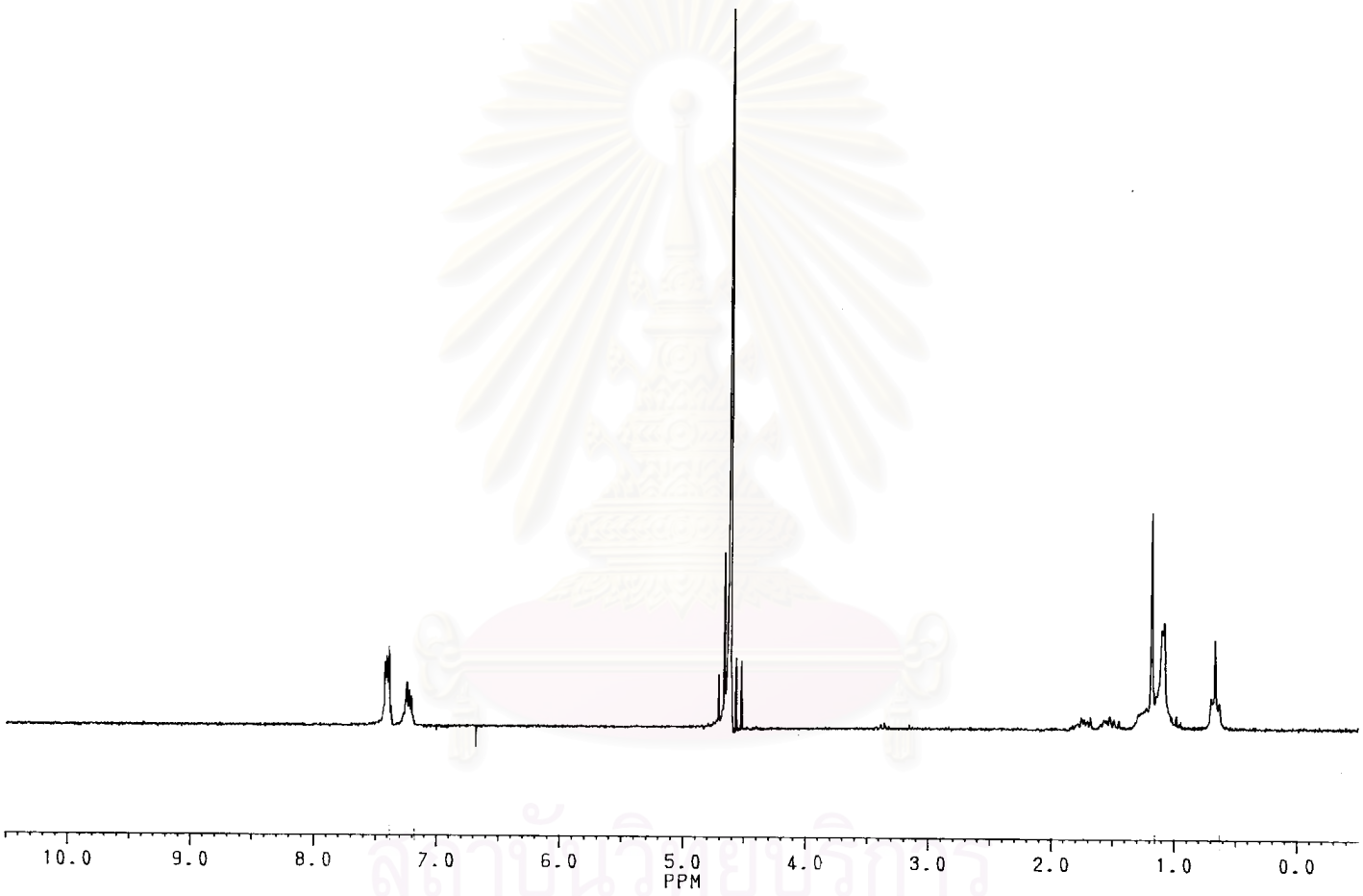

Figure 63: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-phenyl-2-hexyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine. $0.5H_2O$ **(2-20)**

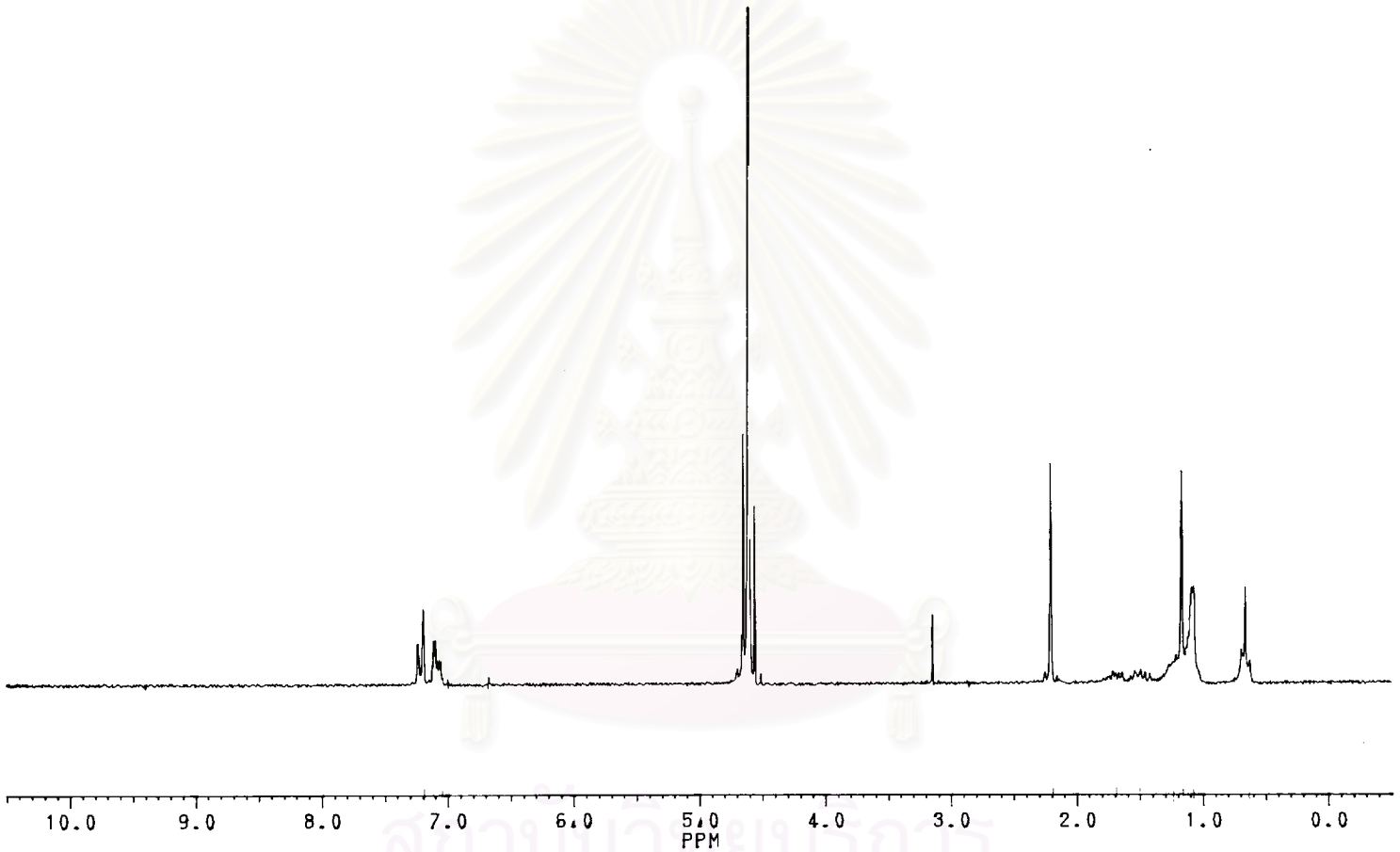

Figure 64: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(4′-methylphenyl)-2-hexyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-21)**

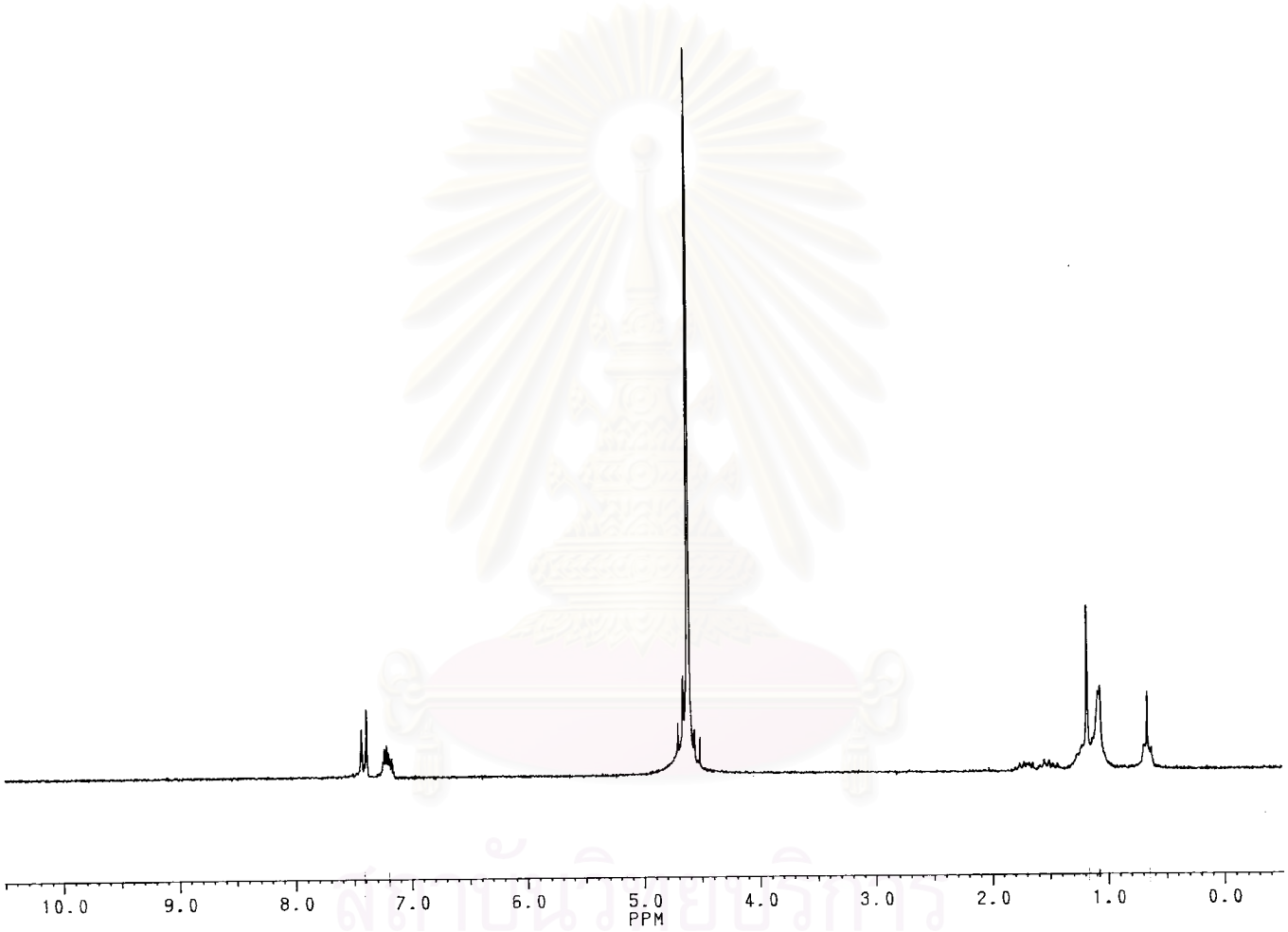

Figure 65: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(4′-ethylphenyl)-2-hexyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-22)**

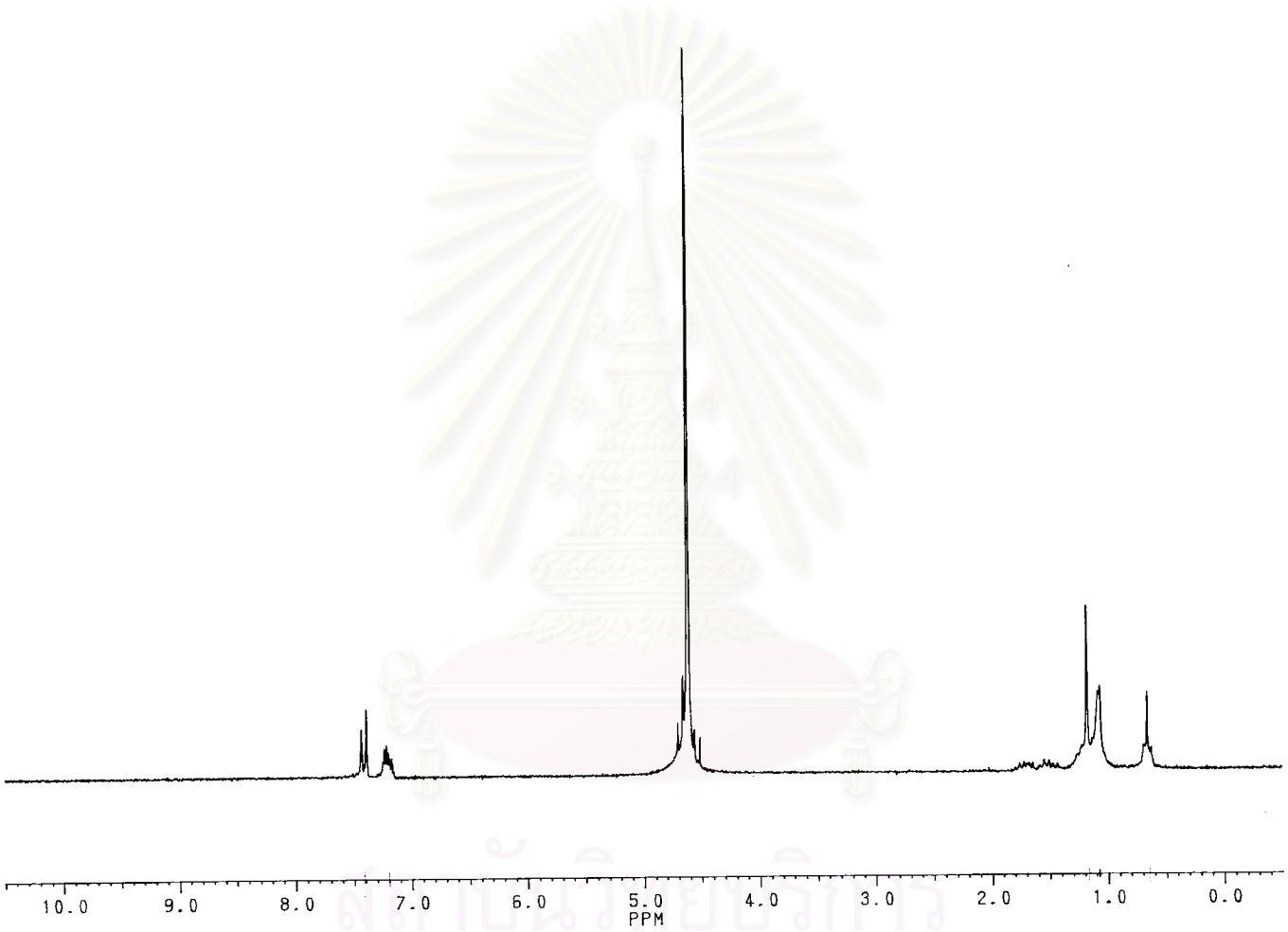

Figure 66: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(4′-chlorophenyl)-2-hexyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-23)**

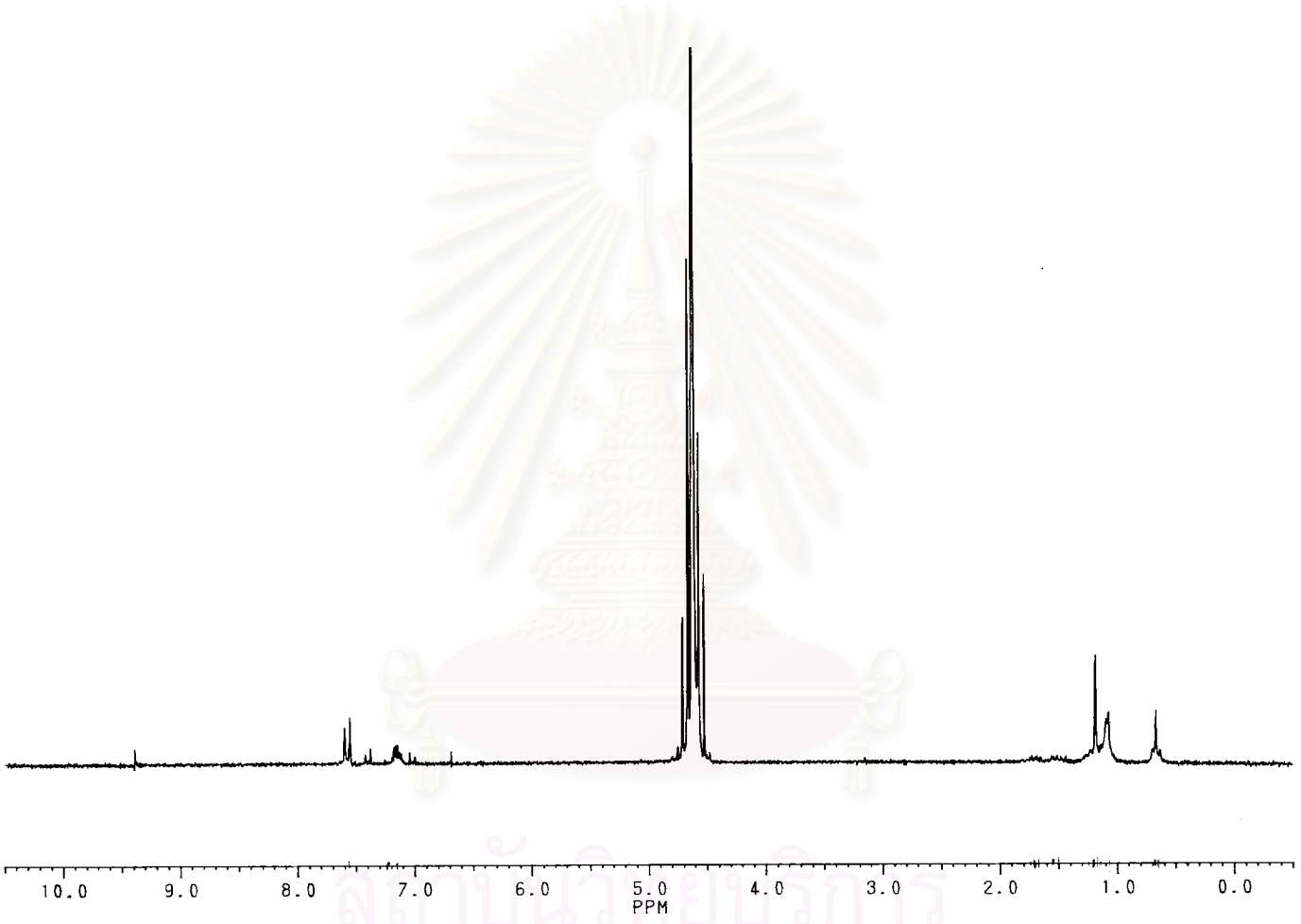

Figure 67: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(4′-bromophenyl)-2-hexyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-24)**

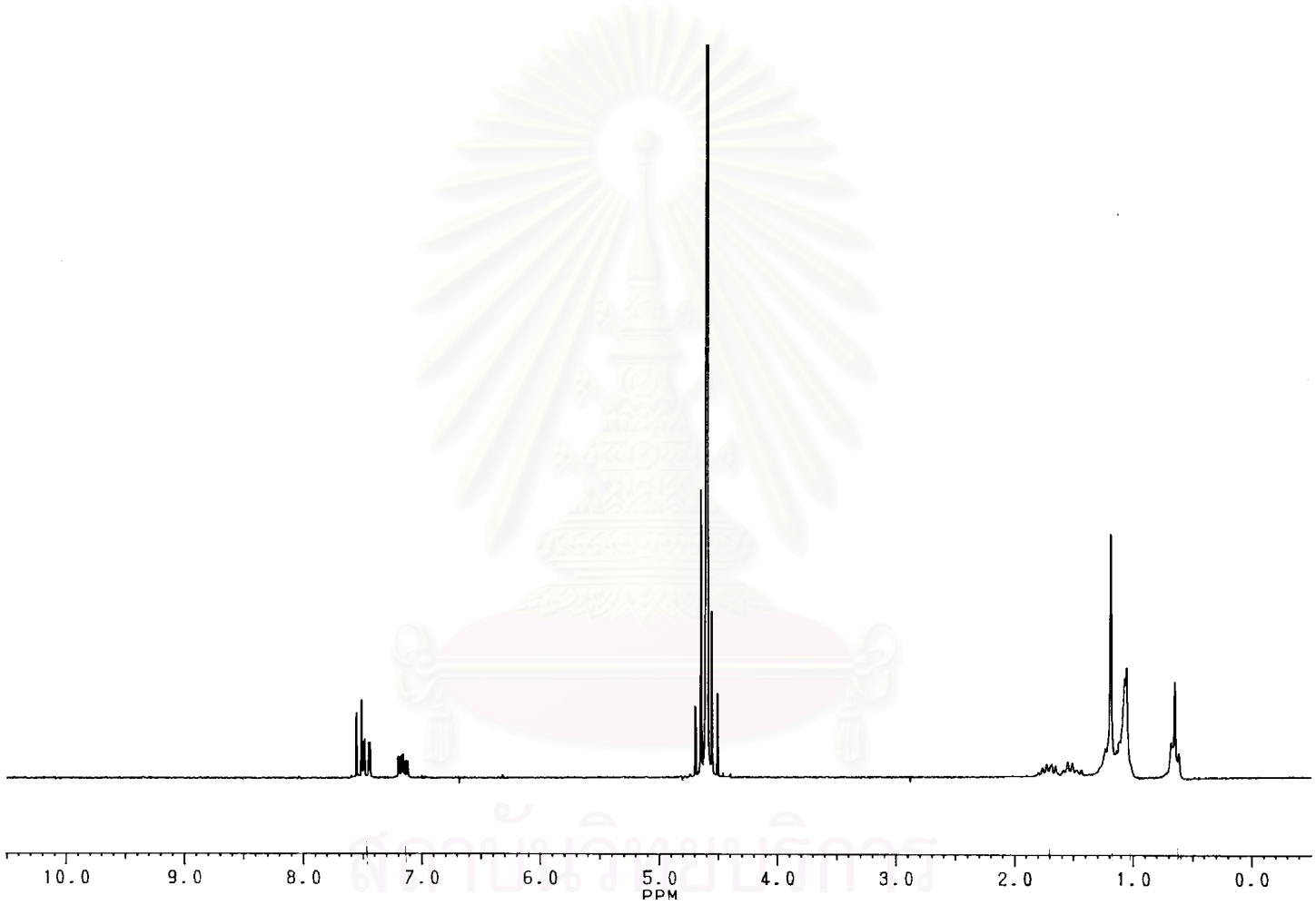

Figure 68: ^{1}H NMR spectrum (D_2O, 200 MHz) 1-(3′,4′-dichlorophenyl)-2-hexyl-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-25)**

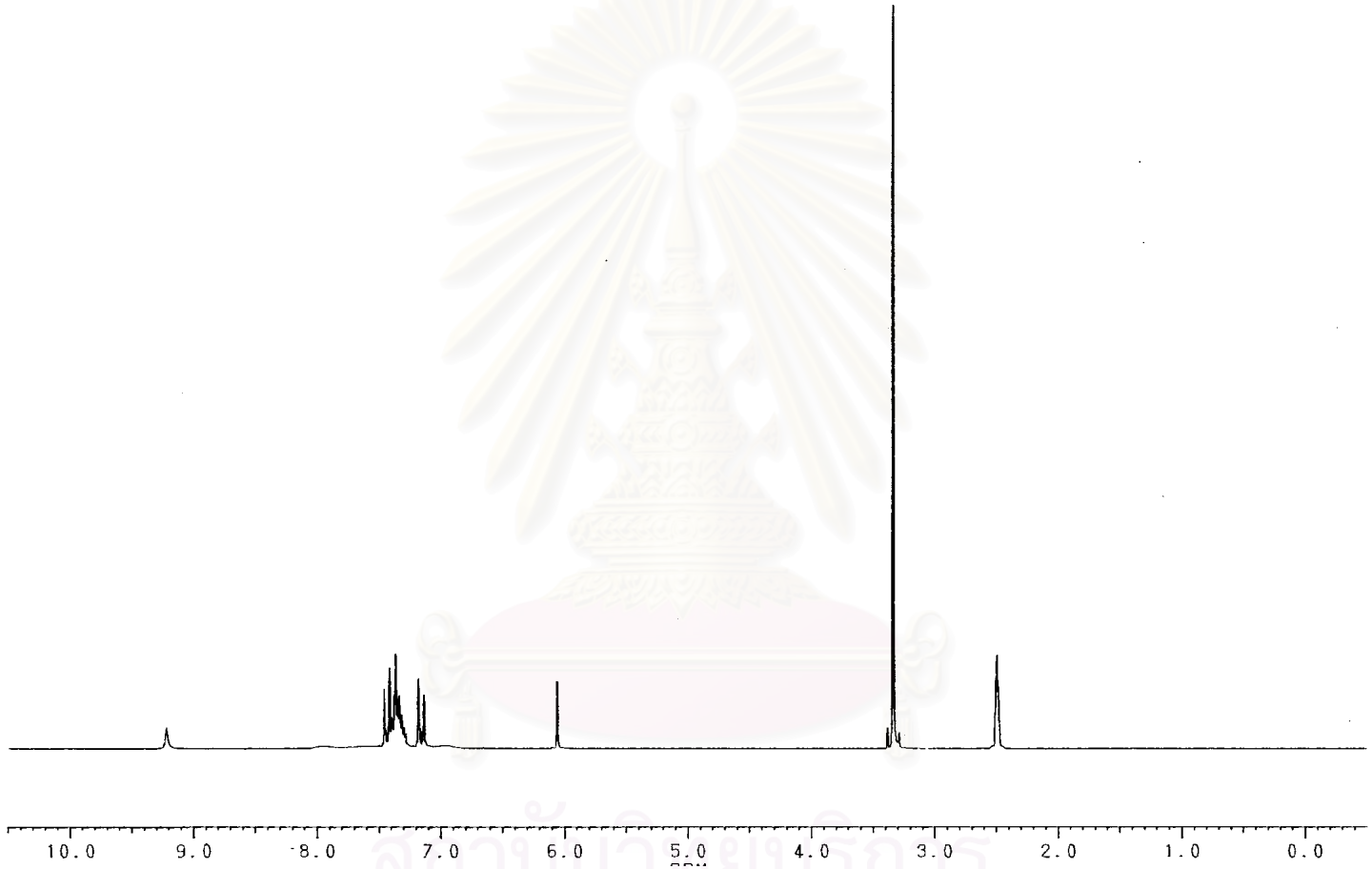

Figure 69: ^{1}H NMR spectrum (DMSO, 200 MHz) 1-(4′-chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-26)**

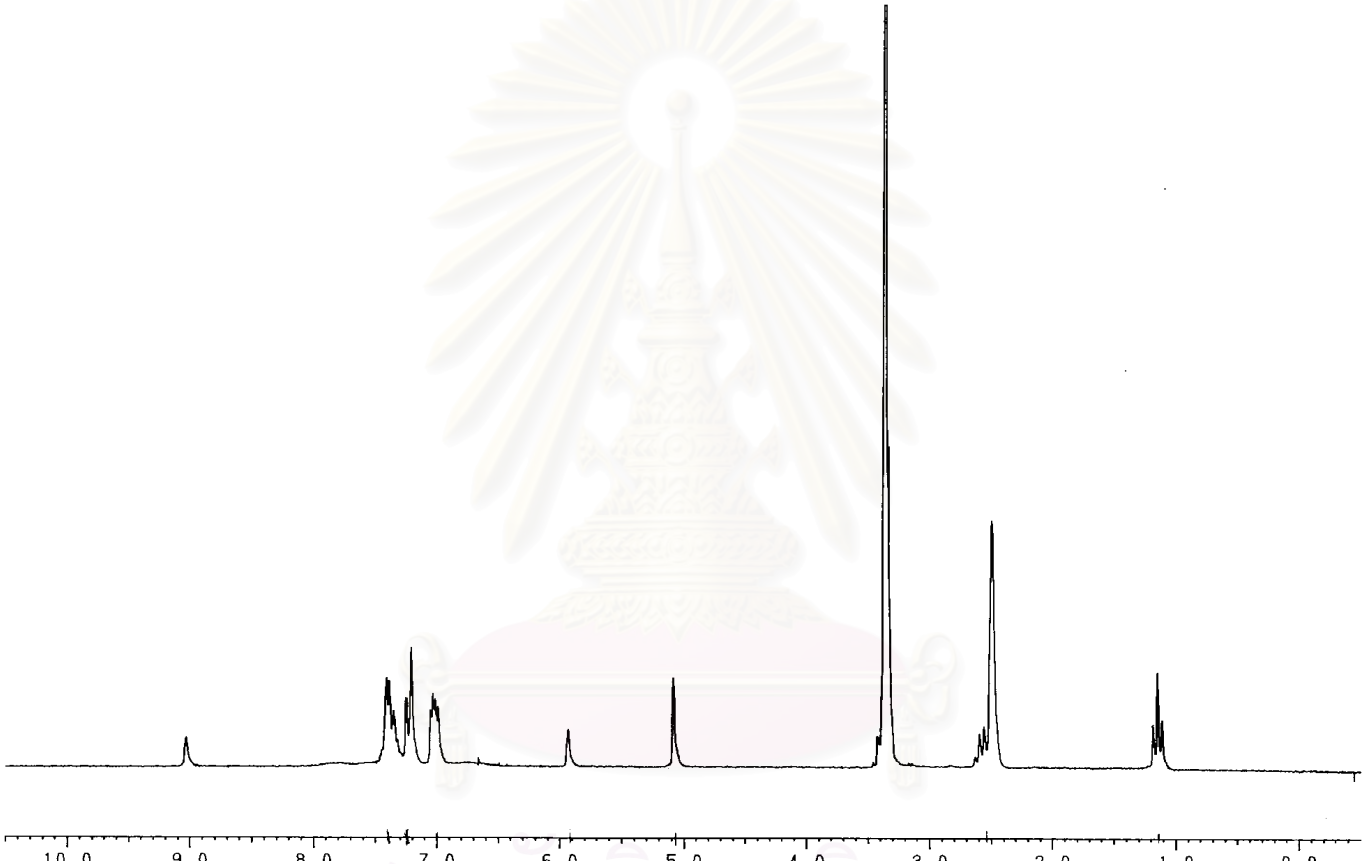

Figure 70: ^{1}H NMR spectrum (DMSO, 200 MHz) 1-(4′-ethylphenyl)-2-(4′′-benzyloxy)phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-27)**

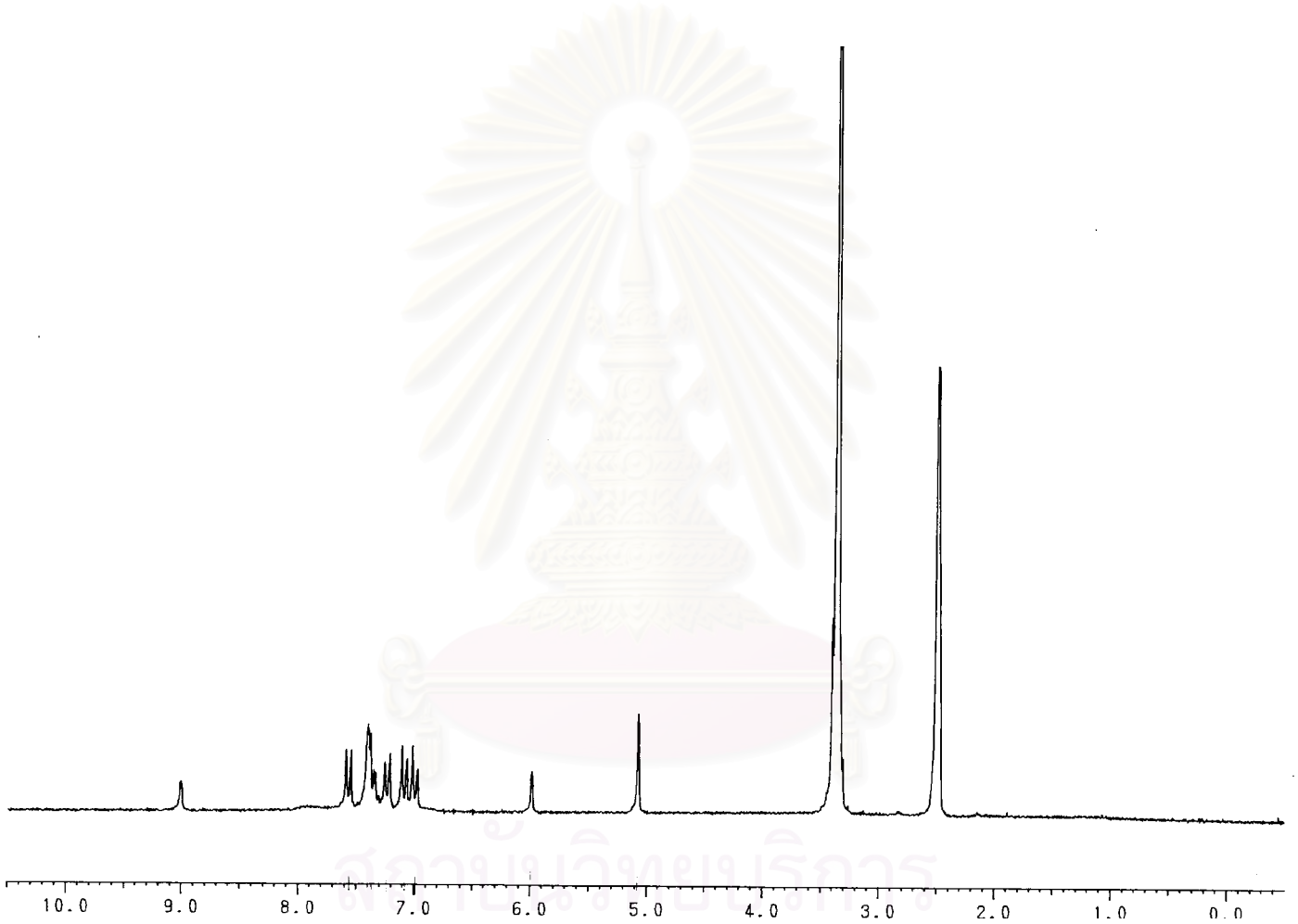

Figure 71: ^{1}H NMR spectrum (DMSO, 200 MHz) 1-(4′-bromophenyl)-2-(4′′-benzyloxy)phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-28)**

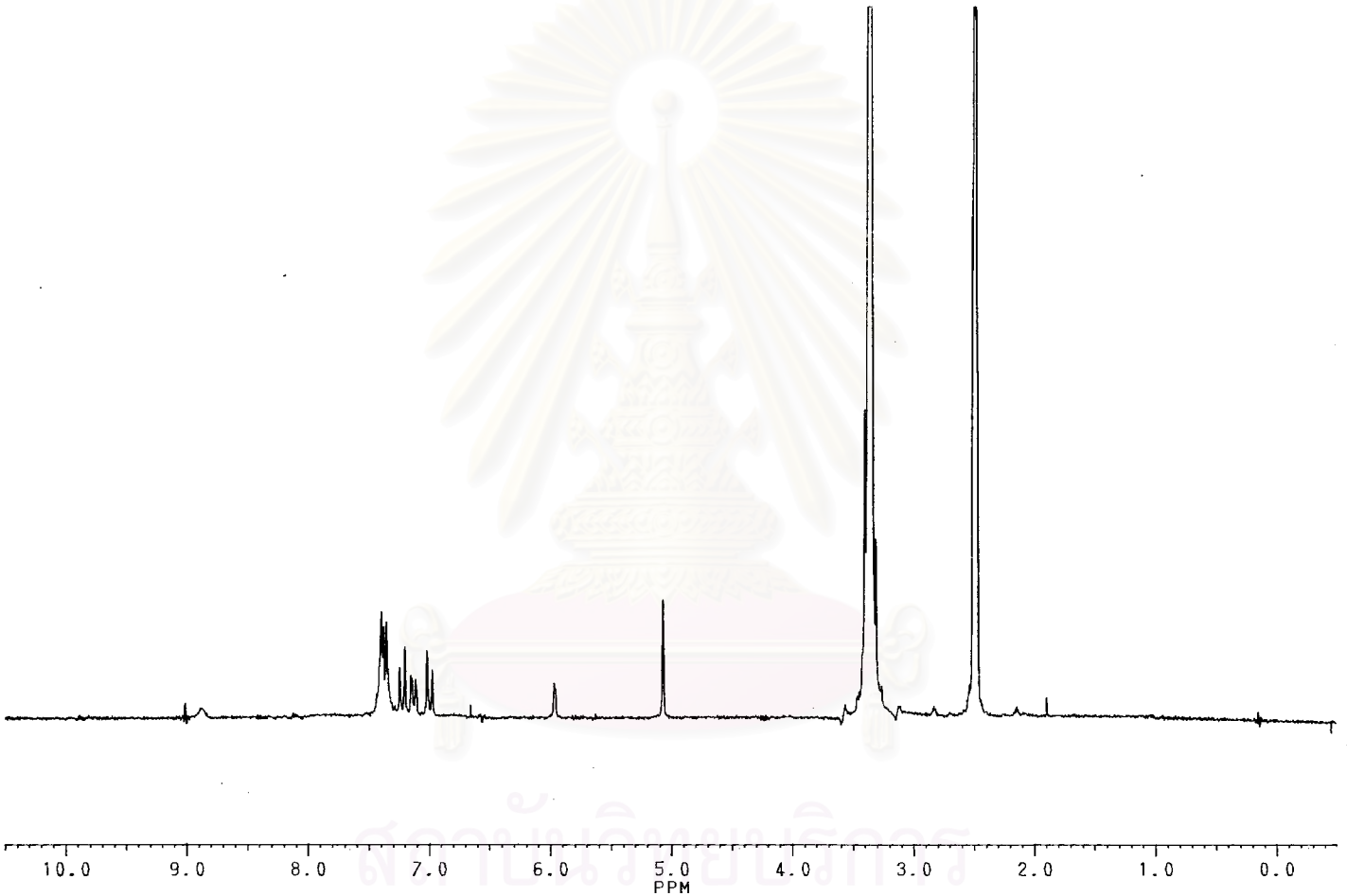

Figure 72: ^{1}H NMR spectrum (DMSO, 200 MHz) 1-phenyl-2-(4′′-benzyloxy)phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-29)**

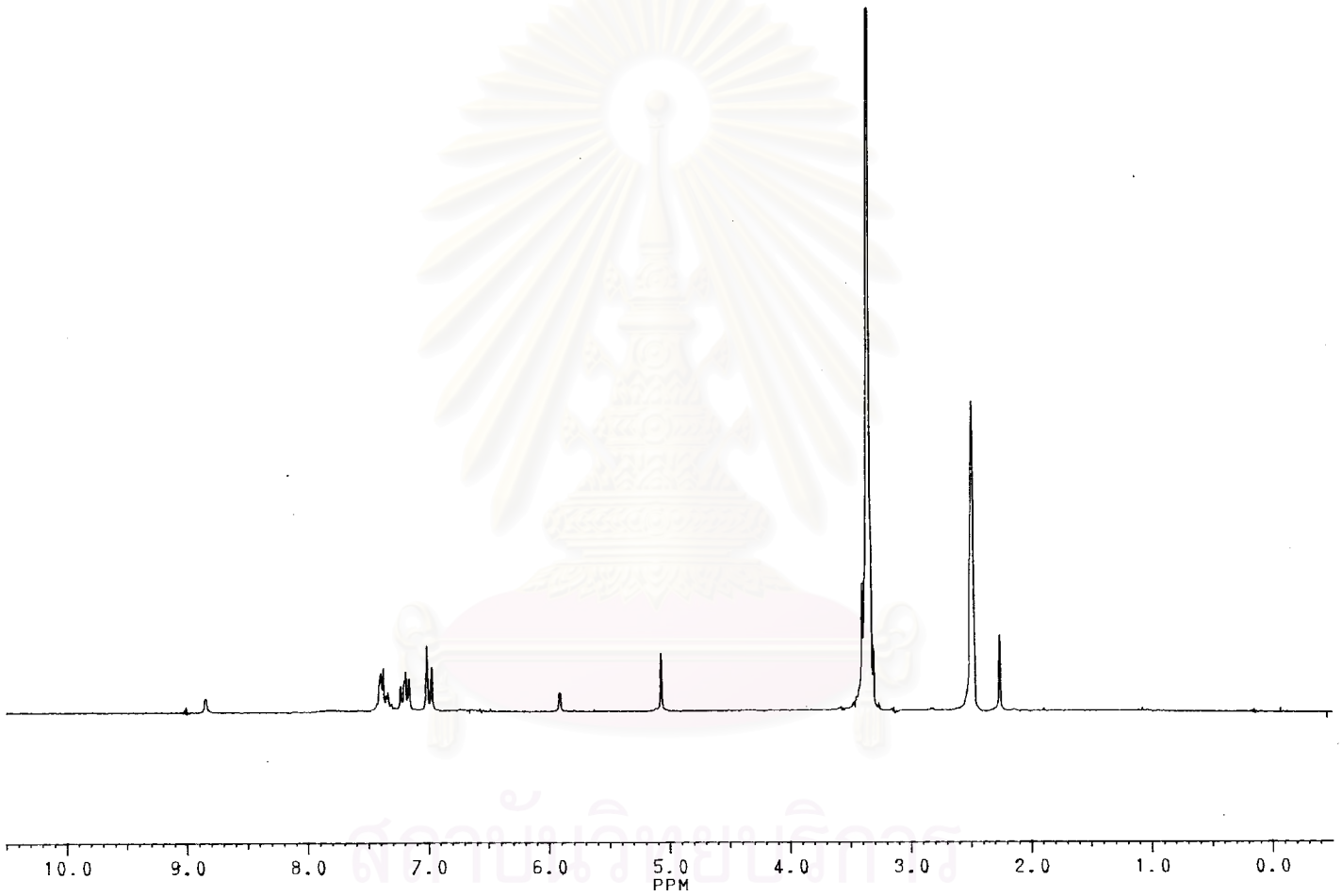

Figure 73: ^{1}H NMR spectrum (DMSO, 200 MHz) 1-(4′-methylphenyl)-2-(4′′-benzyloxy)phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-30)**

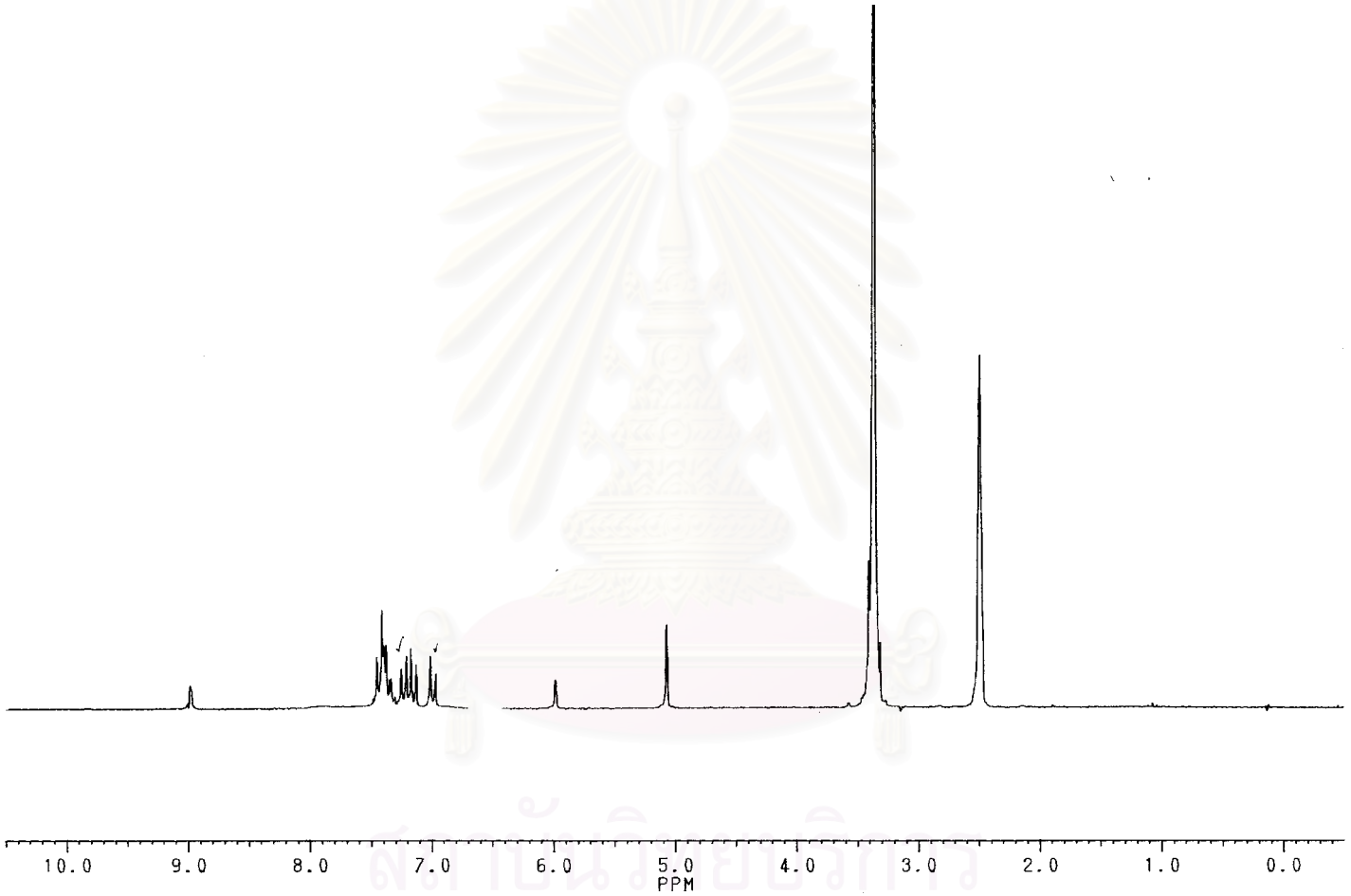

Figure 74: ^{1}H NMR spectrum (DMSO, 200 MHz) 1-(4′-chlorophenyl-2-(4′′-benzyloxy)phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-31)**

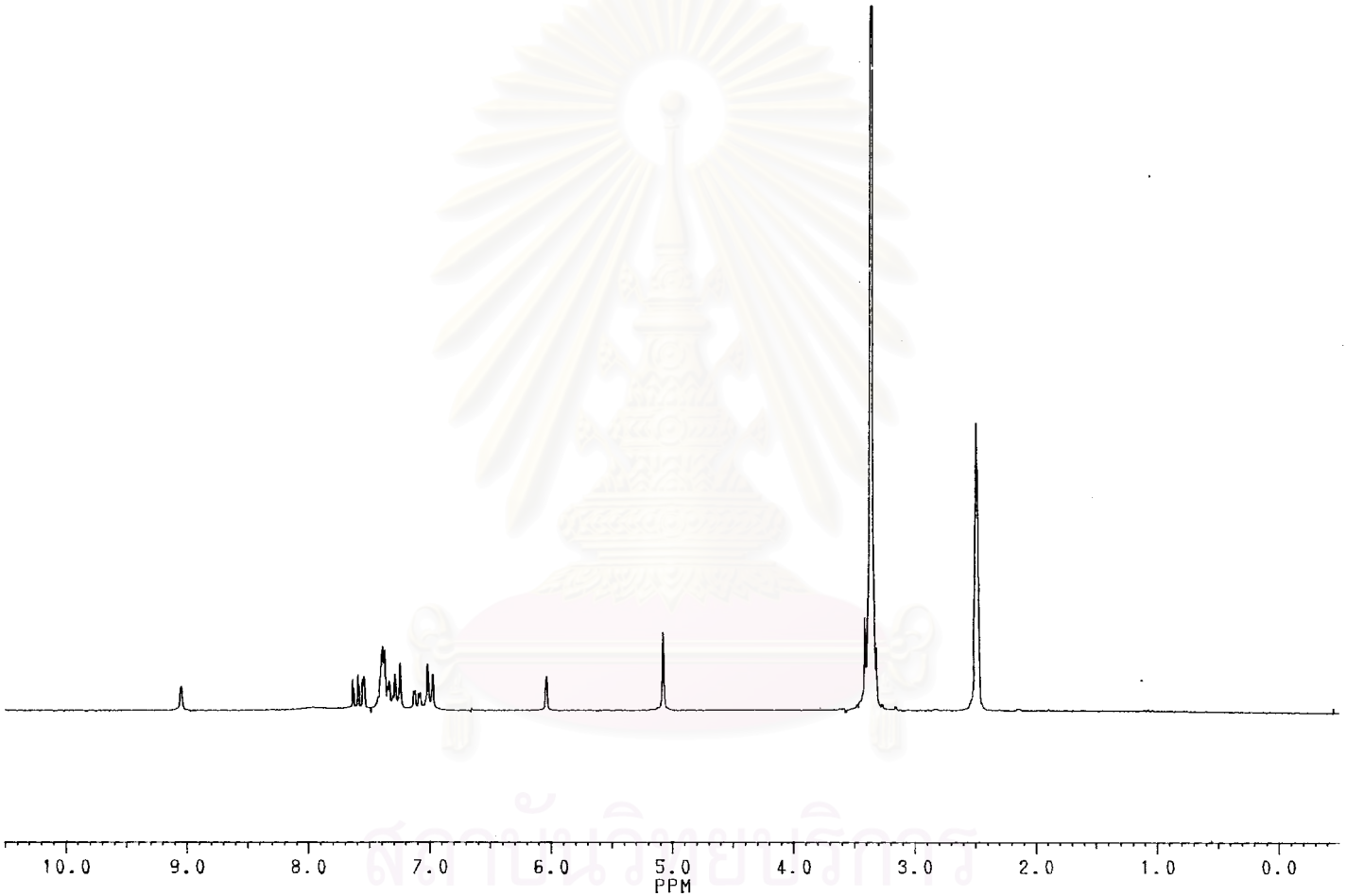

Figure 75: ^{1}H NMR spectrum (DMSO, 200 MHz) 1-(3′,4′-dichlorophenyl-2-(4′′-benzyloxy)phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-32)**

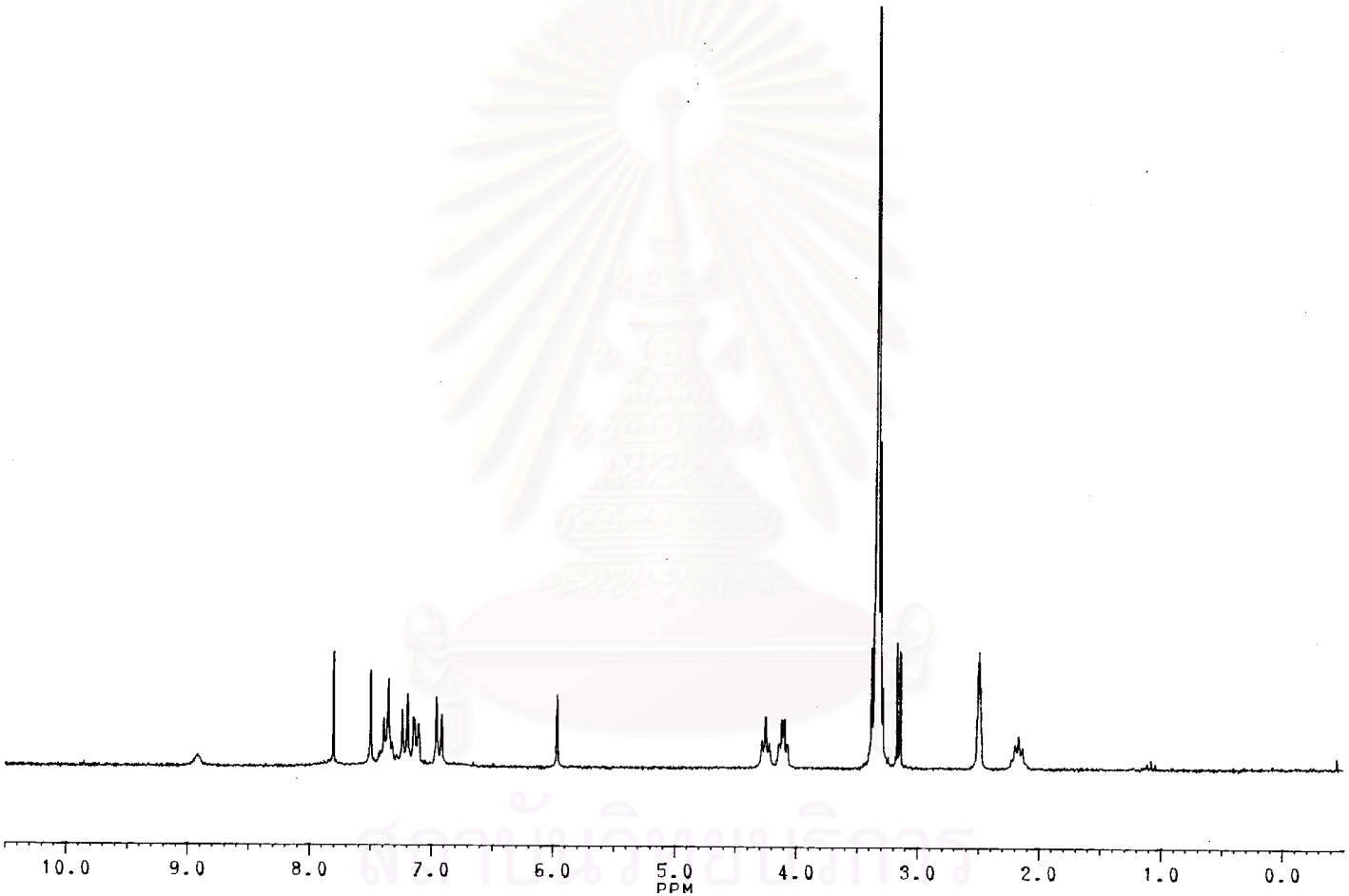

Figure 76: ^{1}H NMR spectrum (DMSO, 200 MHz) 1-phenyl-2-(4′-(2′′,4′′,5′′-trichlorophenoxypropyloxy)phenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-33)**

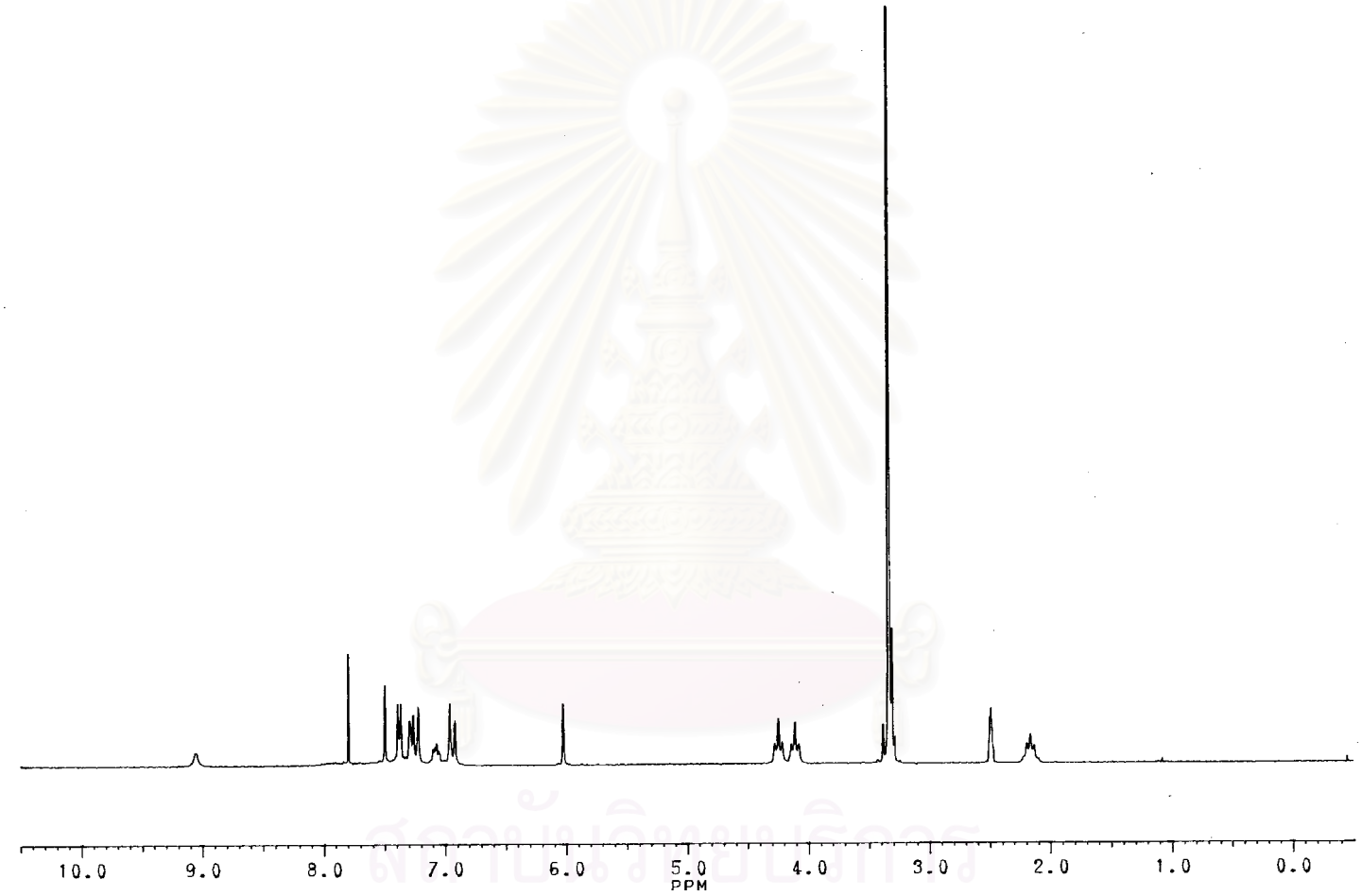

Figure 77: ^{1}H NMR spectrum (DMSO, 200 MHz) 1-(3′-chlorophenyl)-2-(4′-(2′′,4′′,5′′-trichlorophenoxypropyloxy)phenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-34)**

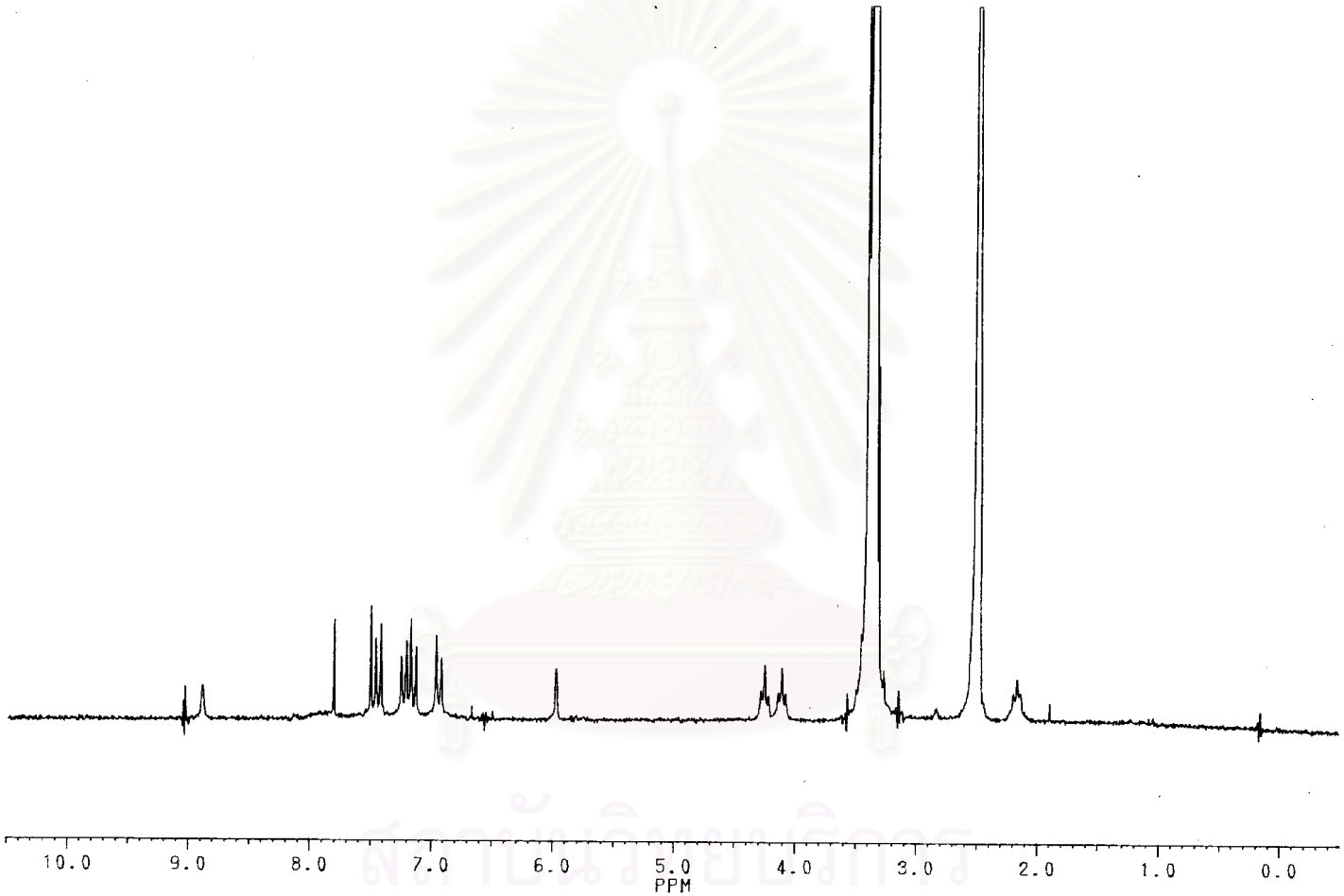

Figure 78: ^{1}H NMR spectrum (DMSO, 200 MHz) 1-(4′-chlorophenyl)-2-(4′-(2′′,4′′,5′′-trichlorophenoxypropyloxy)phenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-35)**

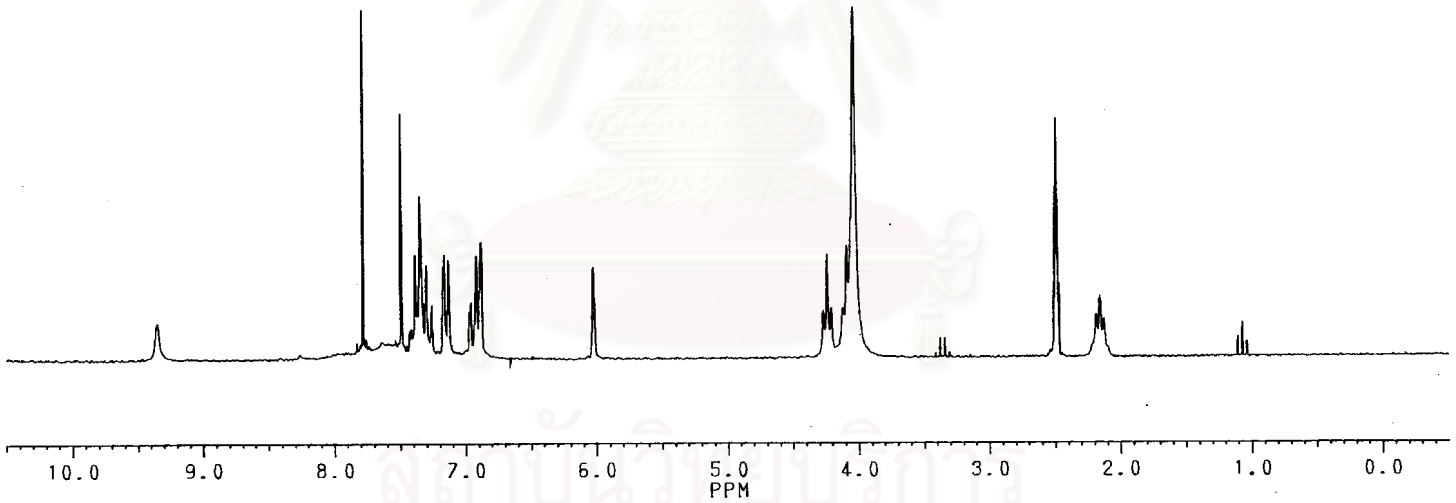

Figure 79: ^{1}H NMR spectrum (DMSO, 200 MHz) 1-phenyl-2-(3′-(2′′,4′′,5′′-trichlorophenoxypropyloxy)phenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-36)**

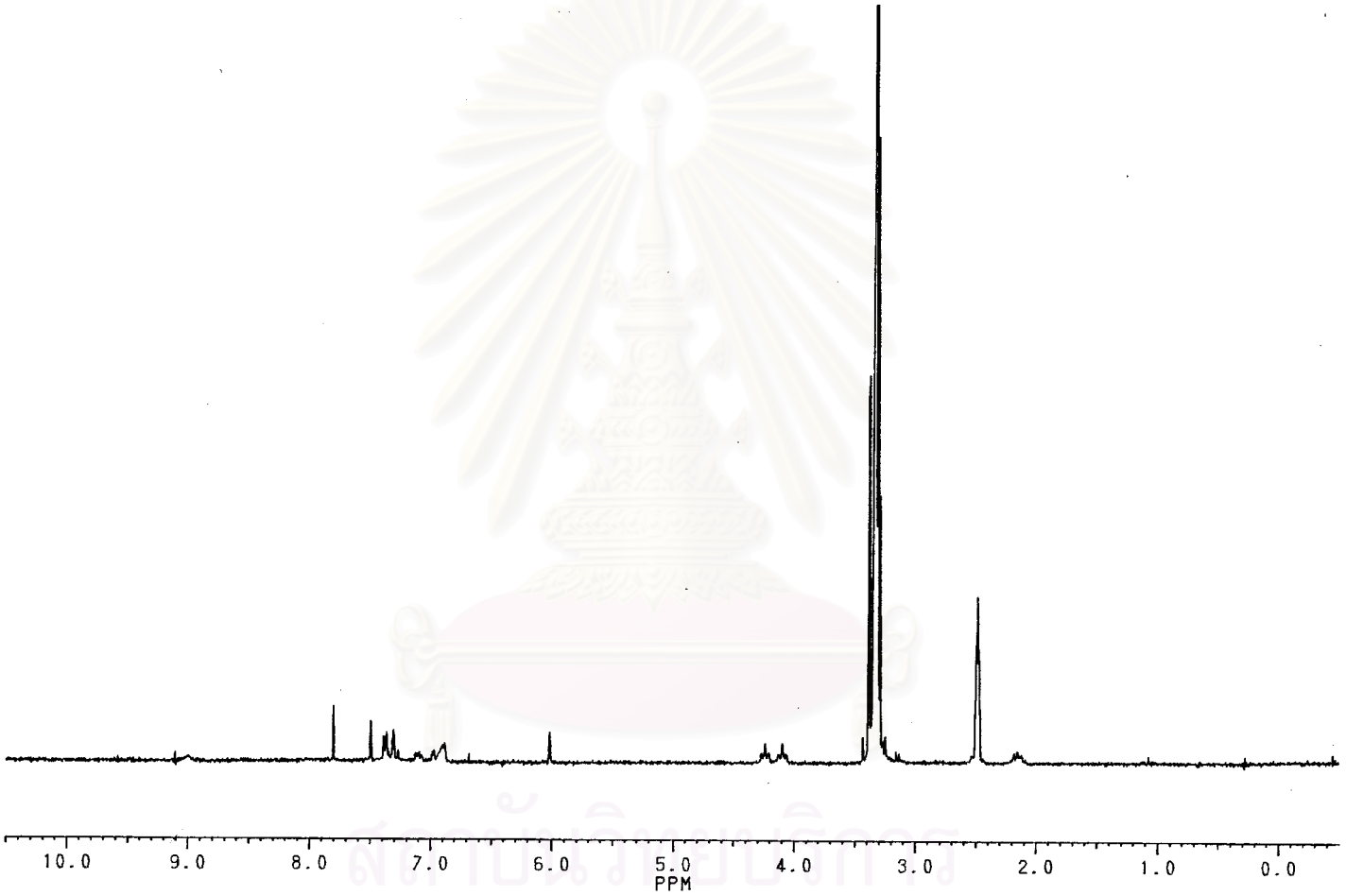

Figure 80: ^{1}H NMR spectrum (DMSO, 200 MHz) 1-(3′-chlorophenyl)-2-(3′-(2″,4″,5″-trichlorophenoxypropyloxy)phenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-37)**

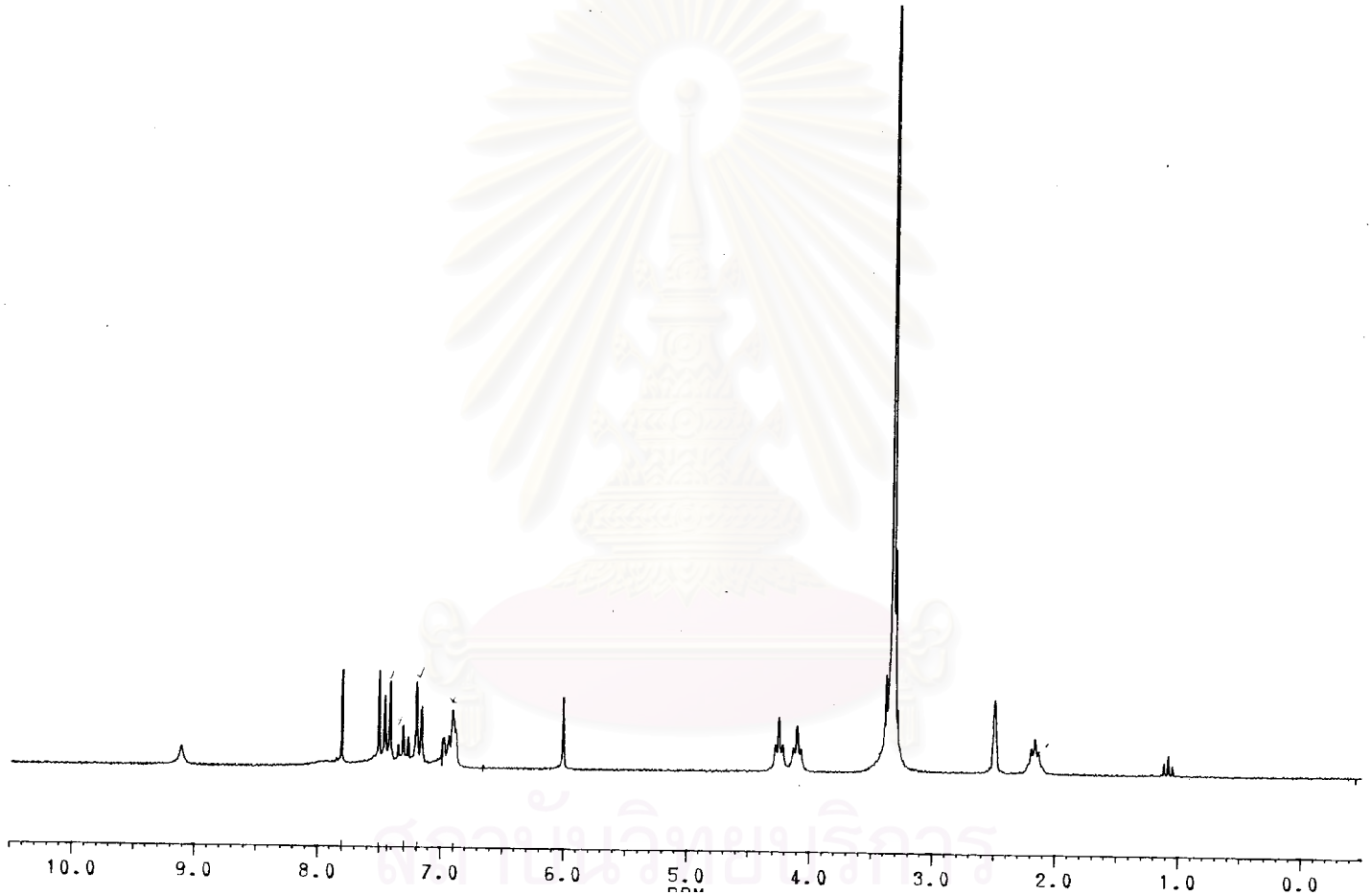

Figure 81: ^{1}H NMR spectrum (DMSO, 200 MHz) 1-(4′-chlorophenyl)-2(3′-(2′′,4′′,5′′-trichlorophenoxypropyloxy)phenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-38)**

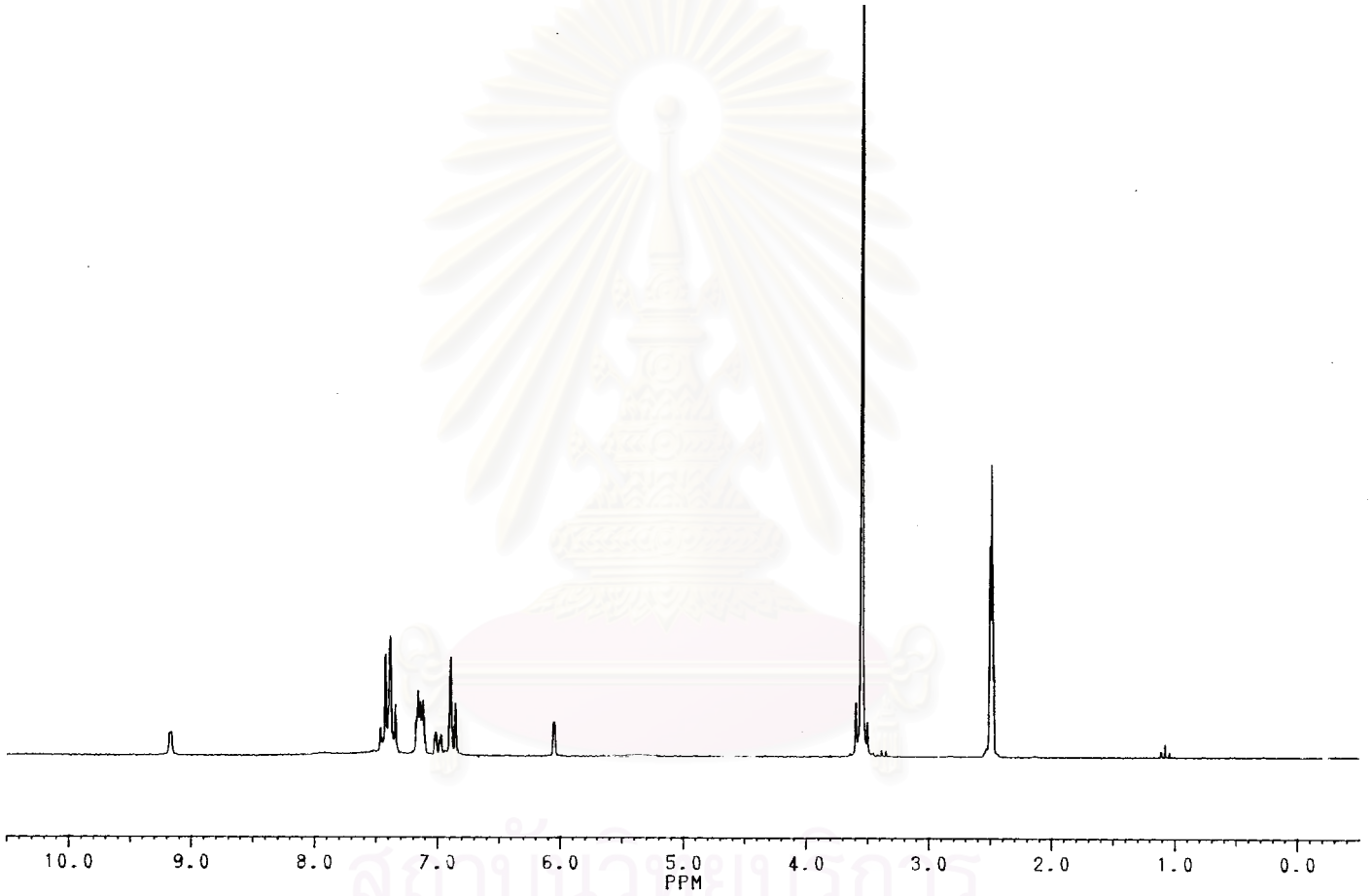

Figure 82: ^{1}H NMR spectrum (DMSO, 200 MHz) of 1-phenyl-2-(3′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-39)**

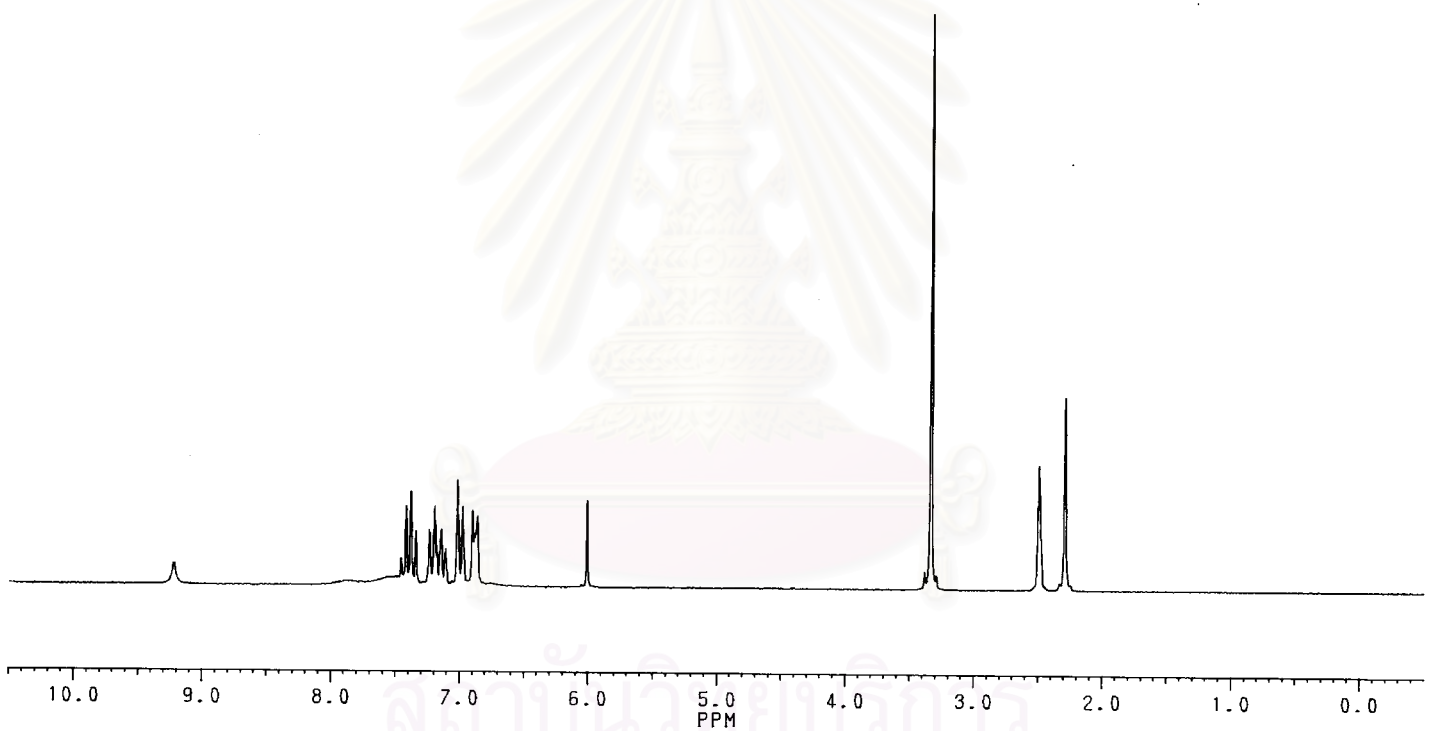

Figure 83: ^{1}H NMR spectrum (DMSO, 200 MHz) of 1-(4′-methylphenyl)-2-(3′′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-40)**

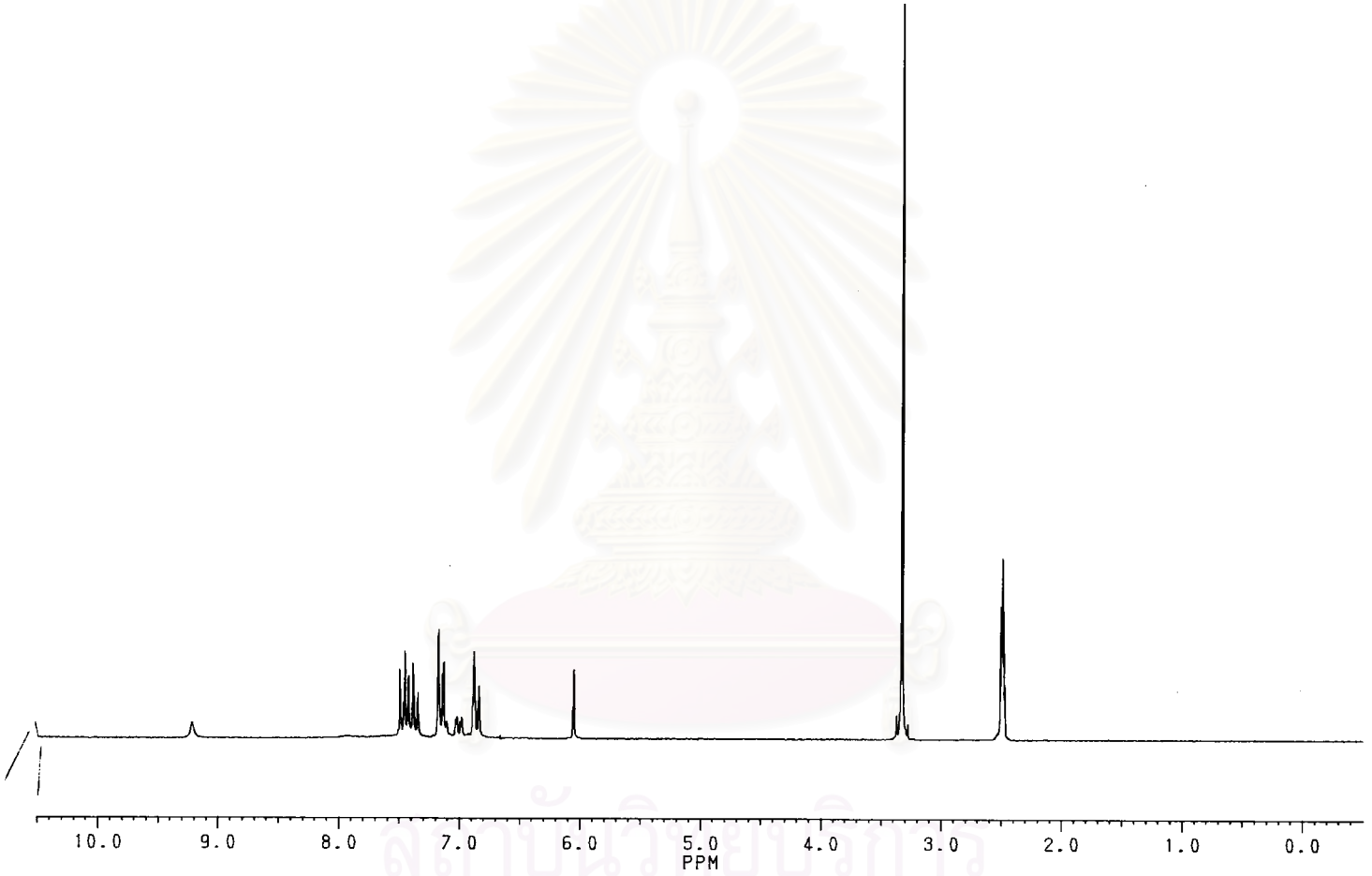

Figure 84: ^{1}H NMR spectrum (DMSO, 200 MHz) of 1-(4′-chlorophenyl)-2-(3′′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-41)**

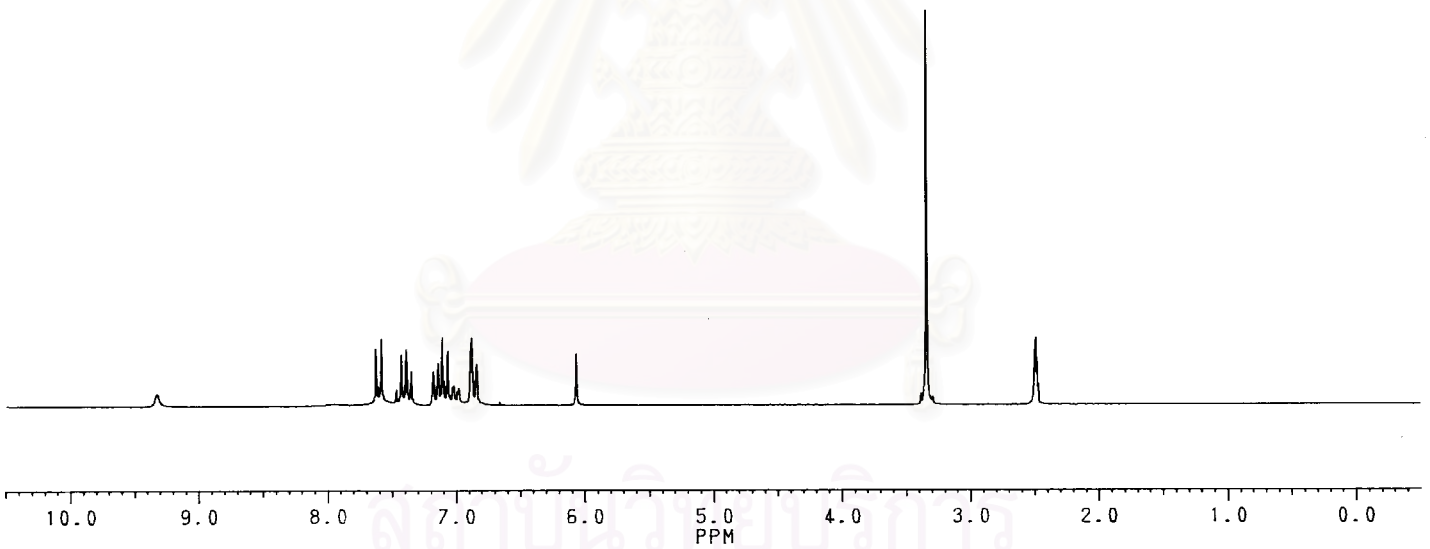

Figure 85: ^{1}H NMR spectrum (DMSO, 200 MHz) of 1-(4′-bromophenyl)-2-(3′′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-42)**

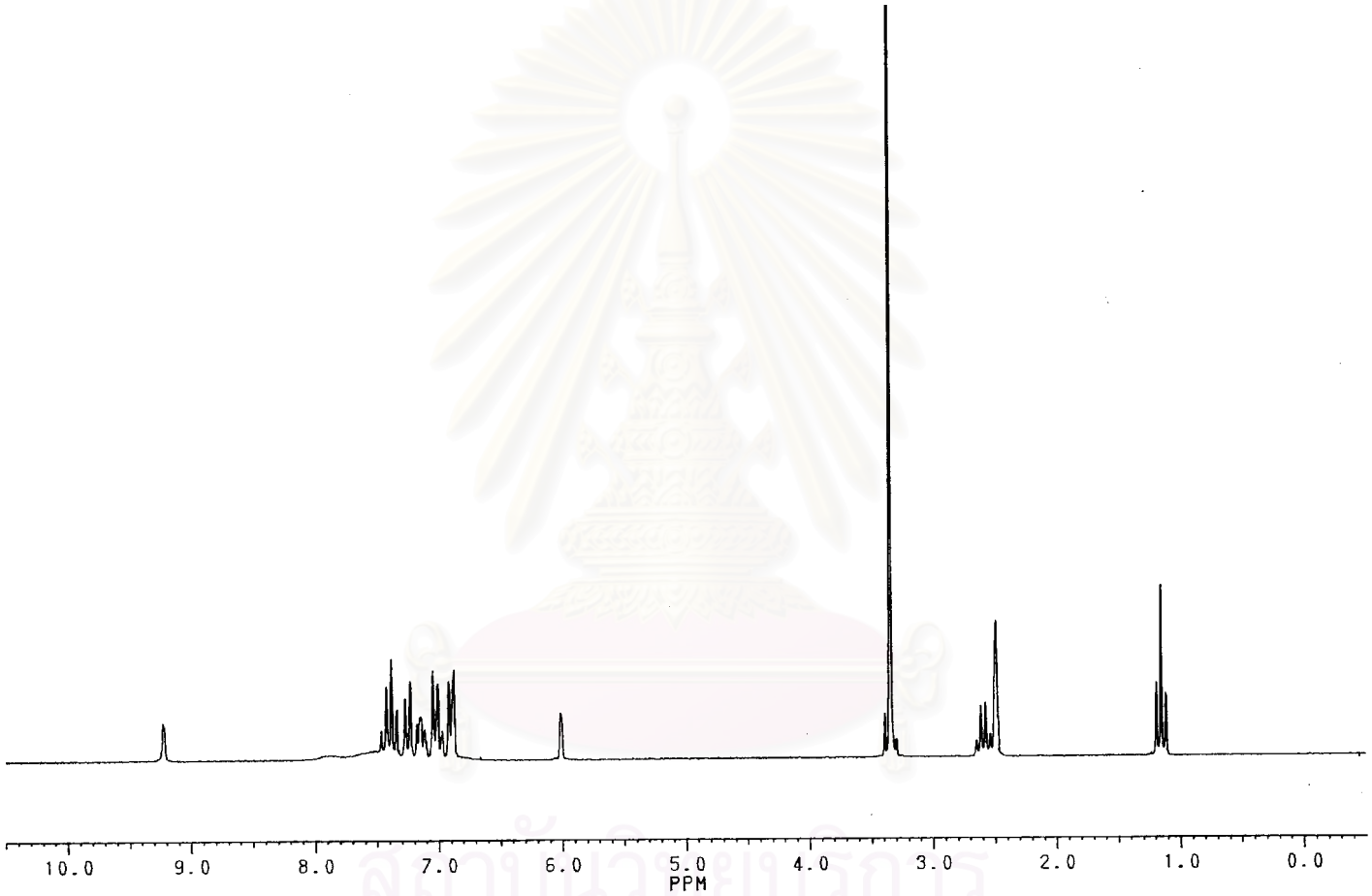

Figure 86: ^{1}H NMR spectrum (DMSO, 200 MHz) of 1-(4′-ethylphenyl)-2-(3′′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-43)**

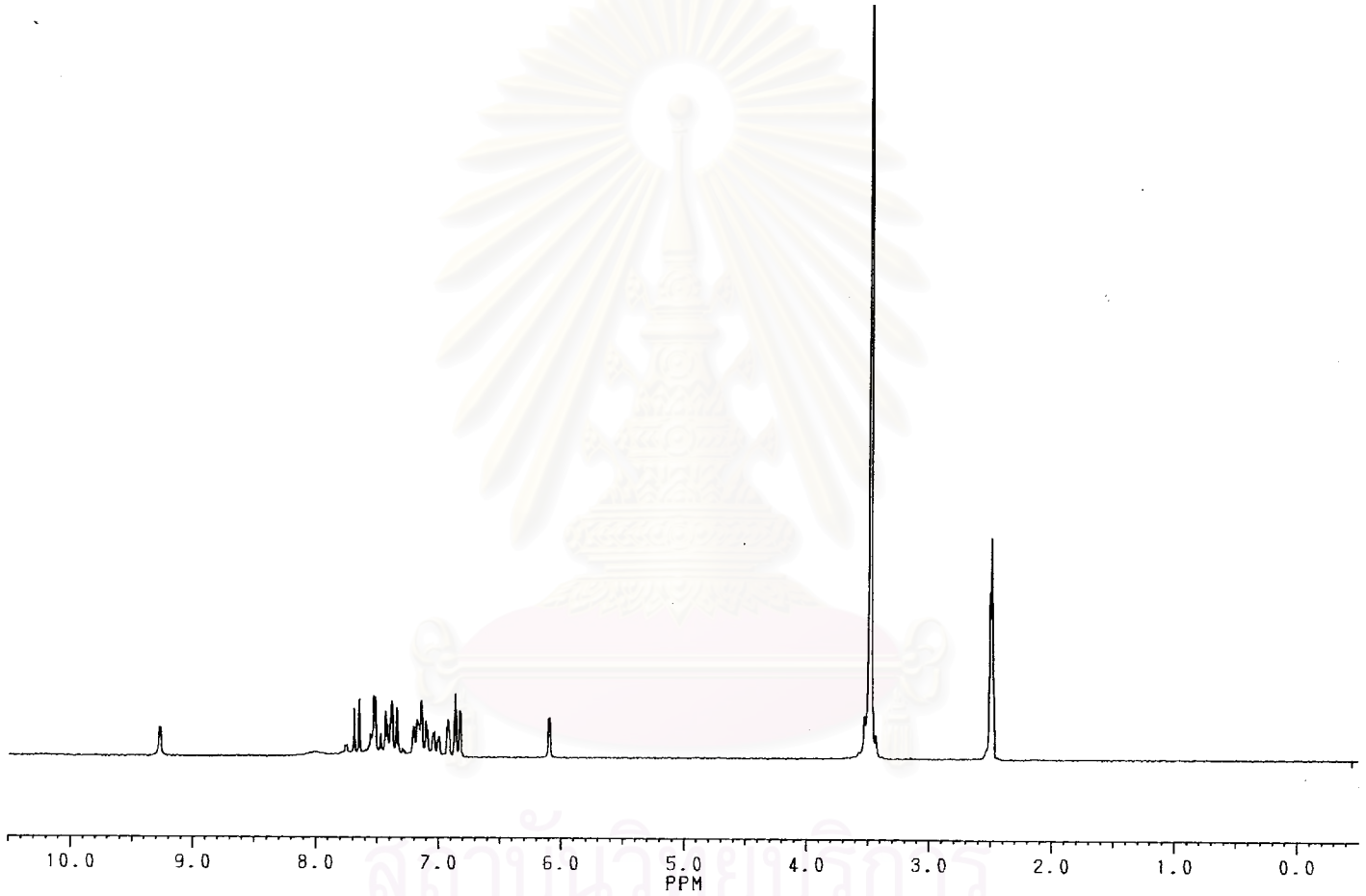

Figure 87: ^{1}H NMR spectrum (DMSO, 200 MHz) of 1-(3′,4′-dichlorophenyl)-2-(3′′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(2-44)**

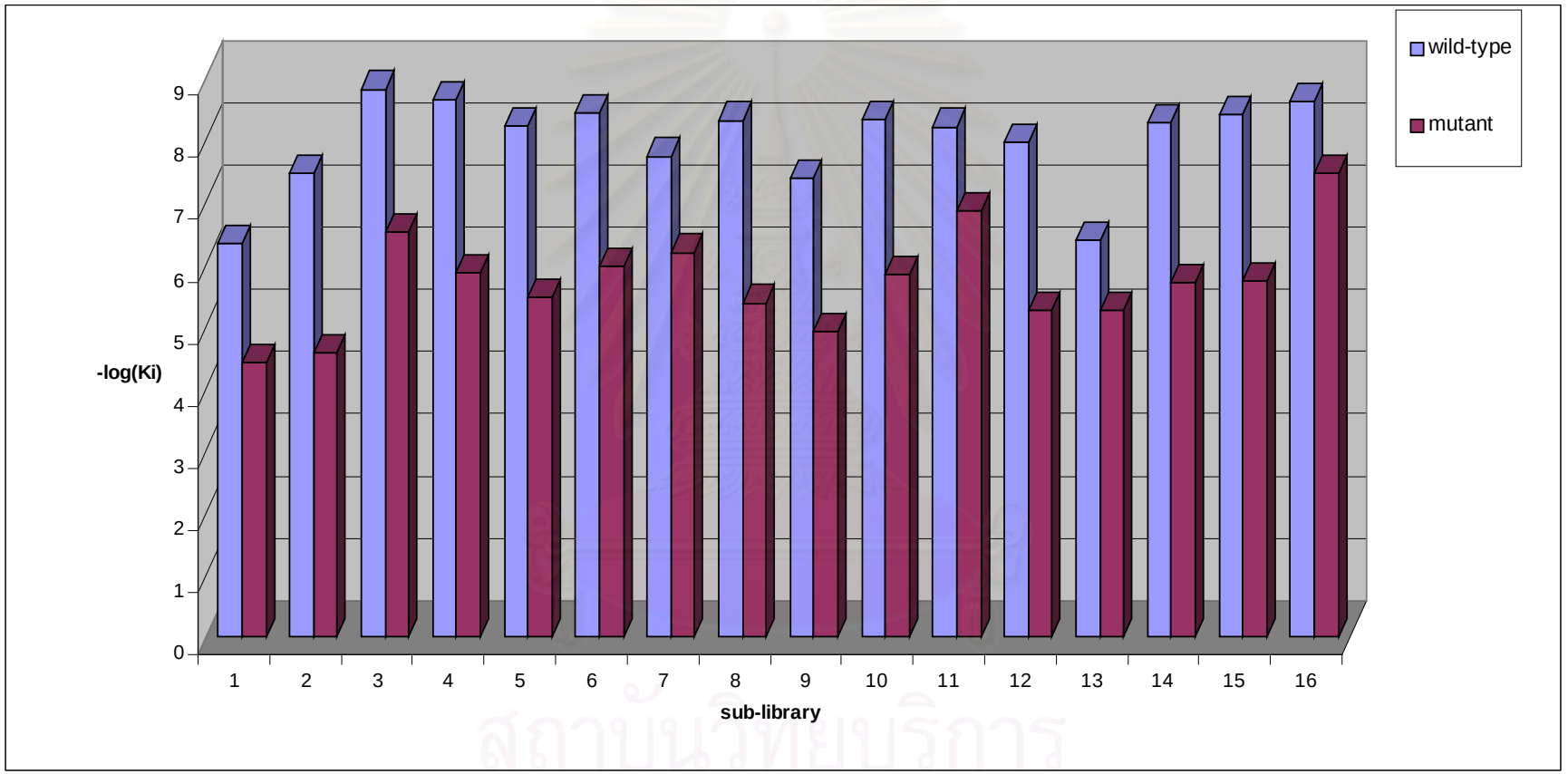

Figure 88: The K_i value of sub-libraries **1-16** against to wild-type and A16VS108T mutant pfDHFRs

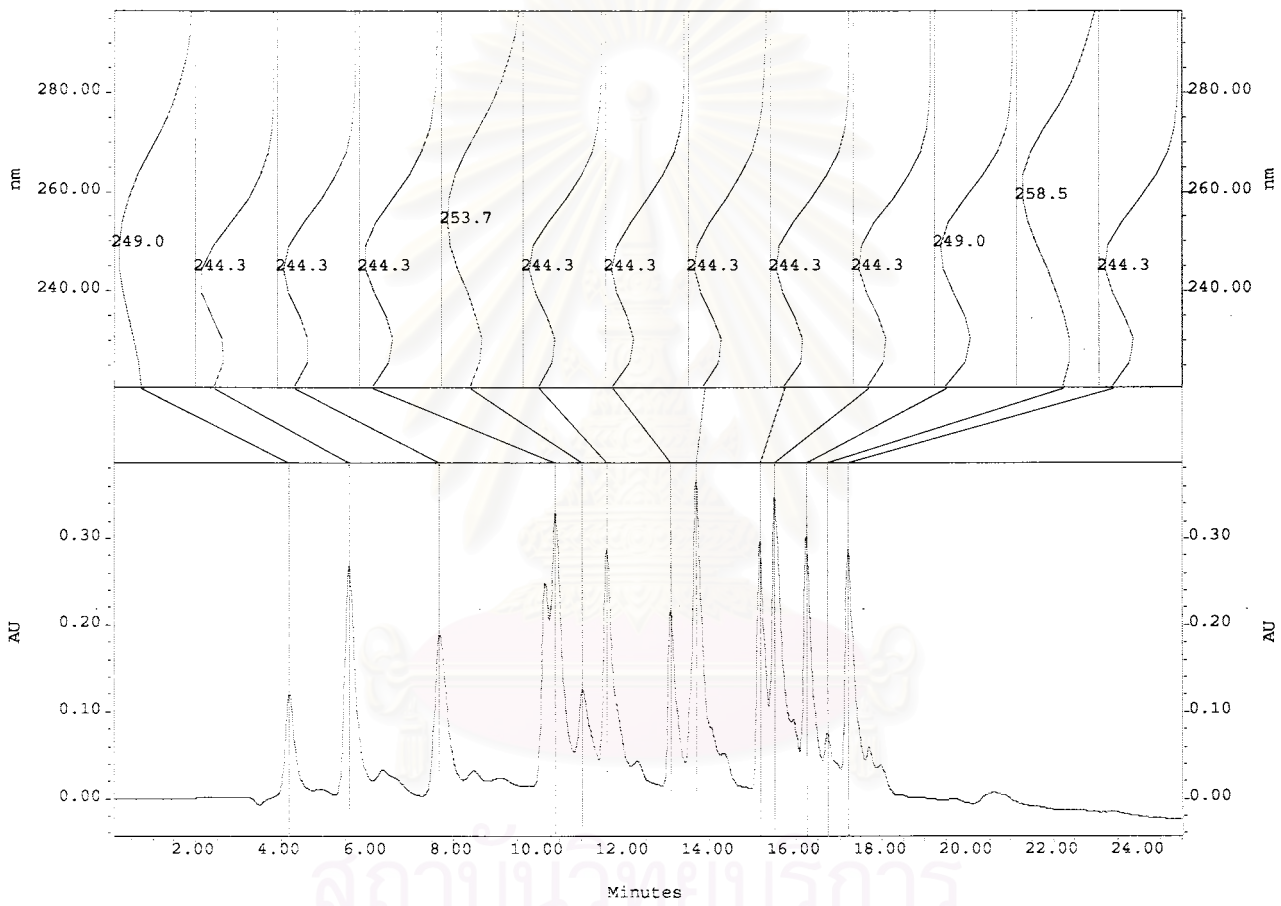

Figure 89: Spectrum and chromatogram from HPLC analysis of reaction between a mixture of 11 ketone compounds and phenylbiguanide hydrochloride; when reaction time as 5 days

VITA

Ms. Neungrutai Saesaengseerung was born on October, 1976 in Bangkok, Thailand. She received the Bachelor Degree of Science in Chemistry from Faculty of Science, Chulalongkorn University in 1998. She enrolled the graduate study in Organic Chemistry Program at Chulalongkorn University for the Master Degree of Science in 1998. She graduated with the Master Degree of Science in 2001.