# *Synthesis of Lamellarins and Related Natural Products*

BY

M. KHALED ARAFEH

A Thesis Presented to the

DEANSHIP OF GRADUATE STUDIES

**KING FAHD UNIVERSITY OF PETROLEUM & MINERALS**

DHAHRAN, SAUDI ARABIA

In Partial Fulfillment of the
Requirements for the Degree of

**MASTER OF SCIENCE**

**In**

CHEMISTRY

March 2009

KINF FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN 31261, SAUDI ARABIA

DEANSHIP OF GRADUATE STUDIES

This thesis, is written by **M.KHALED ARAFEH** under the direction of his advisor and approved by his thesis committee, has been presented to and accepted by the Dean of Graduate Studies, in partial fulfillment of the requirements for the degree of

**MASTER OF SCIENCE IN CHEMISTRY.**

Thesis Committee

Dr. N. ULLAH. (Advisor)

Prof. S.A. ALI. (Member)

Dr. H. AL-MUALLEM. (Member)

Dr. Abdullah J. Al-Hamdan
Department Chairman

Dr. Salam Zummo
Dean of Graduate Studies

27/4/09
[Date]

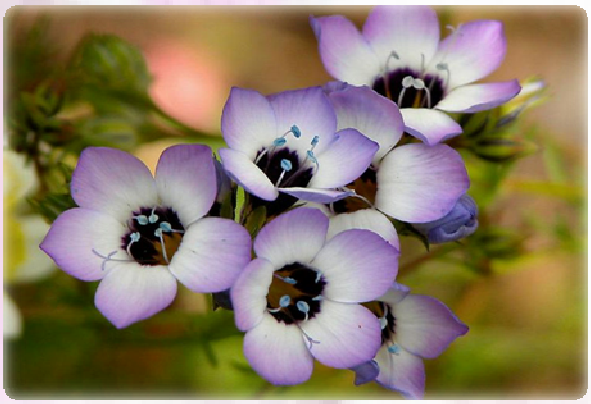
بسم الله الرحمن الرحيم

﴿١٦١﴾ قُلْ إِنَّ صَلَاتِي وَنُسُكِي وَمَحْيَايَ وَمَمَاتِي لِلَّهِ رَبِّ الْعَالَمِينَ ﴿١٦٢﴾ لَا شَرِيكَ لَهُ وَبِذَٰلِكَ أُمِرْتُ وَأَنَا أَوَّلُ الْمُسْلِمِينَ

*Dedicated To*

*My Family and Friends*

# لك عائلـــتي .....



## إلى أبي الغالي .....

إلى من سهر الليالي يدفعنا قدماً إلى الأمام , و كان نبراساً لنا يضيء درب الحياة و قدوة تعلمنا منها الإخلاص و الإتقان ..... لك حبي و إمتناني و تقديري و جزاك الله عنا كل خير

## إلى أمي الغالية .....

إلى من كانت مصدر إلهامنا , فغمرت قلوبنا بحنانها و عطفها و بذلت حياتها من أجلنا . إلى نور عيني و ضياء قلبي ..... لك حبي و إمتناني و تقديري و جزاكي الله عنا كل خير

## إلى أخوتي الأحباء .....

إلى من رافقني درب حياتي , و أجمل لحظات عمري , و كانت لي معهم ذكريات لا تنسى , إلى أخي عبد الرحمن و عامر , و أختي الغالية نور ..... لكم حبي و تقديري , حفظكم الله و رعاكم

# Acknowledgments

All gratitude, praise and glory to Allah Almighty for giving me patience, capability and opportunity to finish this work. Without his Help and Will, nothing is accomplished. Blessing and peace be upon our leader Muhammad, his family, his companions, and those that follow his guidance until the last day.

My deepest appreciation goes to my thesis advisor, Dr.Nisar Ullah, for his invaluable guidance and encouragement. The completion of this work is credited to his tireless support and priceless ideas. His giving me the chance and knowledge to explore the beauty of research can never be forgotten.

My great thanks to my thesis committee members, Dr. Shaikh Asrof Ali and Dr. Hasan Al-Muallem, for their suggestions and valuable comments.

Thanks are due also to the chemistry department and all the faculty members for their encouragement and their direct or indirect help. I am also indebted to the following staff for their invaluable help: Mr. Arab, Mr. Saleem and Mr. Farooqi.

My sincere appreciation goes to my fellow graduate students, brothers who supported me with help and encouragement during the work. Especially Alaeddin Alsbaiee, Mouheddin Alhaffar, Khaled Alhamwi, Nidal Abuthabit, Rami Suleiman, Basem Moosa, Bassem Maythalony, Ahsan Shamsi and Adulrahman asha.

No words can express my gratitude to my wonderful family, My Father, Mother, brothers and little sister. Their encouragements and personal sacrifices are truly appreciated and will be remembered.

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**التخصص:** الكيمياء

**تاريخ التخرج:** آذار 2009

في هذه الدراسة تم إتمام الإصطناع الإجمالي الأول لمركبين طبيعيين من مصادر بحرية و هما نيولامارين أ (Neolamellarin A) مثبط للعامل المحرض على نقص التأكسج ((hypoxia-inducible factor-1 (HIF-1) و مركب أبليسامين 6 (Aplysamine 6) مثبط لأنزيم أيزو برينيل سيستيئين كاربوكسي ميتيل ترانسفيراز ((isoprenylcysteine carboxy methyltransferase (Icmt). أظهرت هذه المركبات الطبيعية فعالية بيولوجية مميزة تجعلها واعدة في مجال الأدوية المضادة للسرطان. إستخدمت استراتيجيات مختلفة لإصطناع هذه المركبات ابتداءاً من مواد أولية شائعة و متوفرة. لإنجاز إصطناع مركب نيولامارين أ (Neolamellarin A) تم تحضير حلقة البيرول مع متبادلاتها الملائمة بإستخدام استراتيجيات مختلفة مثل: طريقة هايزنبرغ (Hinsberg-type pyrrole synthesis), طريقة أريل بيروفيك أسيد (arylpyruvic acid) و طريقة فينغولس أميد (vinylogous amid approach) التي أثبتت نجاعتها كطريقة فعالة لتحضير حلقة البيرول المطلوبة بخطوتين و بمردود تفاعل إجمالي عالي (60%). أتاحت ظروف التفاعل المثالية لأستلة النيتروجين في حلقة البيرول الحصول على الألكوليد نيولامارين أ (Neolamellarin A). تم إصطناع مركب أبليسامين 6 (Aplysamine 6) من خلال تحضير مركبات وسطية متعددة الزمر الوظيفية بطريقة إنتقائية كيميائياً و موضعياً. تضمن سير التفاعل إستخدام مواد أولية شائعة و متوفرة و تم إنجازه في سبع خطوات مع مردود تفاعل إجمالي (55%).

**ماجستير في العلوم الكيميائية**

**جامعة الملك فهد للبترول و المعادن**

**الظهران – السعودية**

**آذار 2009**

# Thesis Abstract

**Name:** **M.Khaled Arafeh**

**Title:** **Synthesis of Lamellarins and Related Natural Products**

**Major:** **Chemistry**

**Date:** **March 2009**

This study accomplished the first total synthesis of two marine natural products neolamellarin A, an inhibitor hypoxia-inducible factor-1 (HIF-1), and Aplysamine 6, an inhibitor of isoprenylcysteine carboxy methyltransferase (Icmt). These natural products have shown potent biological activities and hold promise as anticancer drugs. Different approaches were employed to access these molecules from the common starting materials available in-house. To synthesize neolamellarin A, the pyrrole core with appropriate functionalization required was made by adopting different approaches such as Hinsberg-type pyrrole synthesis, arylpyruvic acid approach and vinylogous amide approach. The latter approach was proved to be an efficient way to prepare the desired pyrrole core in two steps with a high overall yield (60%). The optimized reaction condition for N-acetylation of pyrrole core allowed access to the desired alkaloid neolamellarin A. Aplysamine 6 was synthesized by the preparation of highly functionalized intermediates in a chemo- and regio-selective fashion. The reaction sequence utilized common starting materials available in-house, and the synthesis was accomplished in 7 steps with an overall yield of (55%).

**Master of Science in Chemistry**
**King Fahd University of Petroleum and Minerals**
**Dhahran, Saudi Arabia**
**March 2009**

# CHAPTER 1

# INTRODUCTION AND LITERATURE REVIEW

## 1.1 Introduction to the natural product syntheses

Natural product synthesis, the art and science of constructing the molecules of nature in the laboratory, has been a most attractive area for organic researchers all around the world. The enthusiasm to mimic nature and to discover its secrets, by exploring efficient ways to synthesize novel and important bioactive natural products, pushes the scientists to develop new synthetic methodologies and to explore new chemical reactivity [(1)].

A flagship of organic synthesis[(1)], natural product synthesis is an efficient tool to evaluate the power of existing synthetic methods and the driving force to develop efficient and novel synthetic methods and strategies. The limitless chemical diversity of the natural products provides a huge challenge for synthetic chemists to make these unique structures and cost-effectively in larger quantities to carry out further biological investigations and medical applications [(2)].

As nature remains a source of "leading compounds", natural product synthesis will continue to be the leading science with vast opportunities for new developments and discoveries.

The advances in the drug design and structure-activity relationship studies have allowed modification of the structure of natural products to enhance their potency and improve their selectivity to attain better pharmacological properties [(3)].

## 1.2 Marine Natural Products

The Oceans, with a wide variety of living organisms have been explored since 1943, are now considered to be a great source of potential drugs. The immense marine reservoir provides the natural products' library with many active metabolites which can be an ideal resource for the discovery of new drugs.

Vast and unique organic chemical structures have been observed in the extracts of marine organisms. Many of them have been shown to be promising candidates as anti-cancer agent as well as anti-infective agent (4). Cytarabine (Ara C) is used efficiently in cancer chemotherapy, for the treatment of leukemia, and it was developed from marine origin natural products[5] (spongothymidine and spongouridine) (Figure 1).

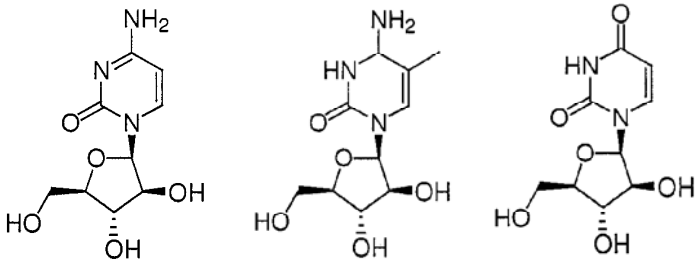



**Figure 1**

Sponges have proved to be the richest source of marine natural products with interesting biomedical potentials. Organic compounds synthesized by both sponges and microorganisms living within the sponge host were found to possess potent anticancer, antibiotic, antifungal, antiviral, antiparasitic, and antifouling activities [6] .

During the search for novel and new anti-cancer drugs, enormous work has been carried out in order to screen the crude extracts of marine organisms for biological activity. That research has led to many promising findings such as antitumor and/or cytotoxic activities. Further efforts have to be focused on translating these bioactivities into useful drugs by using effective pre-fractionation strategies, advanced identification techniques, and novel synthetic methods [7].

## 1.3 Lamellarins

### 1.3.1 Isolation and Structure

Lamellarins, a family of alkaloids bearing 3,4-diarylpyrrole unit, have been isolated from marine prosoblanch mollusks, ascidians, and sponges. The first isolation of Lamellarins (A, B, C, D) from Lamellaria sp., a prosobranch mollusk (Family *Lamellariidae*) was in 1985[8]. Later, four additional analogues, lamellarins (E-H), were isolated and identified by Fenical and co. workers in 1988 [9].

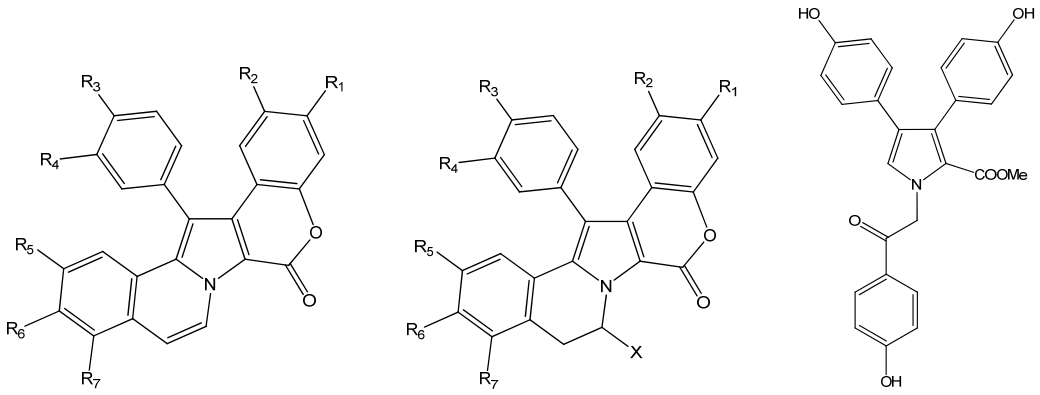
In 1993, Bowden's group reported six new Lamellarins, Lamellarins (I-M) and the triacetate of lamellarin N, isolated from an Australian colonial ascidian *Didemnum* sp[10]. In the following year, Capon and colleagues reported the first two lamellarins with unfused pyrrole ring, namely lamellarins O and P [11]. The instability of both compounds prevented further biological studies and restricted the exploration of the bioactivity of this new class of lamellarins. One year later, two new stable members of this class, lamellarins Q and R, were reported by the same research group [12]. Among them lamellarin O has shown a good potential as an effective cytotoxic agent for leukemia and lymphoma.

The first optically active Lamellarin, Lamellarin S, was isolated in 1996 by Urban and Capon. Lamellarins displayed a positive optical rotation due to the atropisomerism, which is induced by the restricted rotation of the C-1 phenyl substituent around the C1-C11 bond[13].

The alphabet continues with lamellarins T, U, V, W, and Y, which were isolated in their 20-sulfate form[14]. Among the sulfated members of lamellarins family, Lamellarin α 20-sulfate was the best known metabolite. It was isolated from an unidentified ascidian collected from the Arabian Sea coast of India, and it was proved to be an interesting inhibitor of HIV-1 integrase[15].

So far more than 30 members belonging to the Lamellarins family and their related pyrrole-derived alkaloids have been isolated and identified [16].

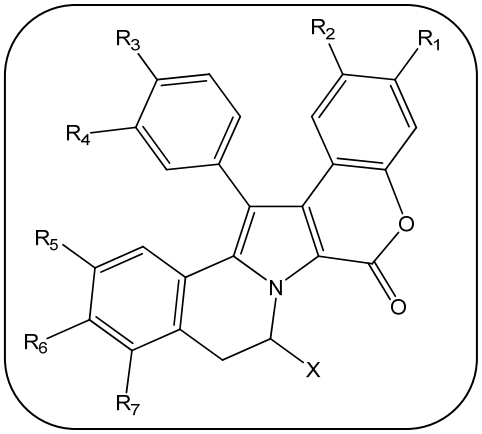
All the lamellarins and their related alkaloids have a general structure skeleton composed of pyrrole core with polyoxygenated aromatics on their periphery. Based on the nature of the pyrrole core, they can be classified into two main groups i.e; as a fused lamellarins **(1)** and **(2)** or unfused lamellarins **(3)**. The former can be subdivided further into two main classes, **(1)** bearing a double bond on C5-C6 position or **(2)** a single bond at the same position.




**1** **2** **3**

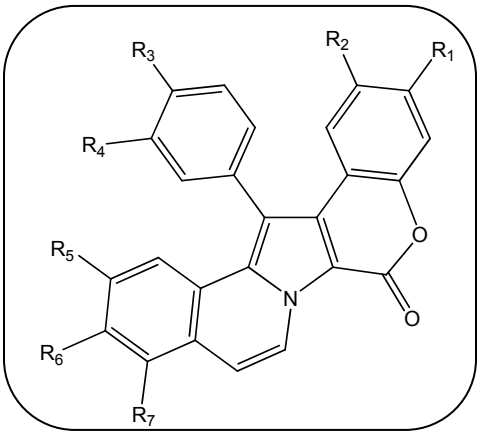
Over 30 members belonging to the lamellarin family share the same pentacyclic core with structural variation coming from the hydroxy and methoxy substitution pattern of the aryl rings. In some cases, one of the phenols is sulfated, resulting in sulfated lamellarins with interesting biological activity (Table 1) (Table 2) (Figure 2).

Other interesting structural features appear from the geometry of the molecule. The presence or absence of a double bond in C5-C6 position results in planar or nonplanar pentacyclic chromophore (6H-[1]benzopyrano[4,3:4,5]pyrrolo[2,1-α]isoquinolin-one). The aromatic ring linked to the C1 position is disposed orthogonally to the pentacyclic system, and the restricted rotation around the C1-C11 bond induces an atropisomerism whose energy barrier depends on the substitution pattern of the molecule.




| *Lamellarin* | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | $R_7$ | $X$ |
|---|---|---|---|---|---|---|---|---|
| *A* | OH | OMe | OH | OMe | OMe | OMe | OMe | OH |
| *C* | OH | OMe | OH | OMe | OMe | OMe | OMe | H |
| *E* | OH | OMe | OMe | OH | OMe | OMe | OH | H |
| *F* | OH | OMe | OMe | OMe | OMe | OMe | OH | H |
| *G* | OMe | OH | OMe | OH | OMe | OH | H | H |
| *I* | OH | OMe | OMe | OMe | OMe | OMe | OMe | H |
| *J* | OH | OMe | OMe | OMe | OMe | OH | H | H |
| *K* | OH | OMe | OH | OMe | OMe | OMe | OH | H |
| *L* | OH | OMe | OMe | OH | OMe | OH | H | H |
| *S* | OH | OH | OH | OH | OMe | OH | H | H |
| *T* | OH | OMe | OMe | OH | OMe | OMe | OMe | H |
| *U* | OH | OMe | OMe | OH | OMe | OMe | H | H |
| *V* | OH | OMe | OMe | OH | OMe | OMe | OMe | OH |
| *Y* | OH | OMe | OMe | OH | OH | OMe | H | H |
| *Z* | OMe | OH | OH | OH | OMe | OH | H | H |
| *β* | OH | OH | OMe | OH | OH | OH | H | H |
| *γ* | OH | OMe | H | OMe | OMe | OMe | OH | H |
| *χ* | OAc | OMe | OAc | OMe | OMe | OAc | H | H |

**Table 1**




| *Lamellarin* | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | $R_7$ |
|---|---|---|---|---|---|---|---|
| *B* | OH | $OCH_3$ | OH | $OCH_3$ | $OCH_3$ | $OCH_3$ | $OCH_3$ |
| *D* | OH | $OCH_3$ | OH | $OCH_3$ | $OCH_3$ | OH | H |
| *H* | OH | OH | OH | OH | OH | OH | H |
| *M* | OH | $OCH_3$ | OH | $OCH_3$ | $OCH_3$ | $OCH_3$ | OH |
| *N* | OH | $OCH_3$ | $OCH_3$ | OH | $OCH_3$ | OH | H |
| *W* | OH | $OCH_3$ | $OCH_3$ | OH | $OCH_3$ | $OCH_3$ | $OCH_3$ |
| *X* | OH | $OCH_3$ | $OCH_3$ | OH | $OCH_3$ | $OCH_3$ | H |
| *α20-sulfate* | $SO_3Na$ | $OCH_3$ | $OCH_3$ | OH | $OCH_3$ | $OCH_3$ | H |
| *Є* | OH | OMe | OMe | OMe | OMe | OMe | OH |
| *ζ* | OH | OMe | OMe | OMe | OMe | OMe | OMe |
| *η* | OH | OMe | OMe | OMe | OMe | OMe | H |
| *Φ* | OAc | OMe | OAc | OMe | OAc | OMe | OMe |

**Table 2**

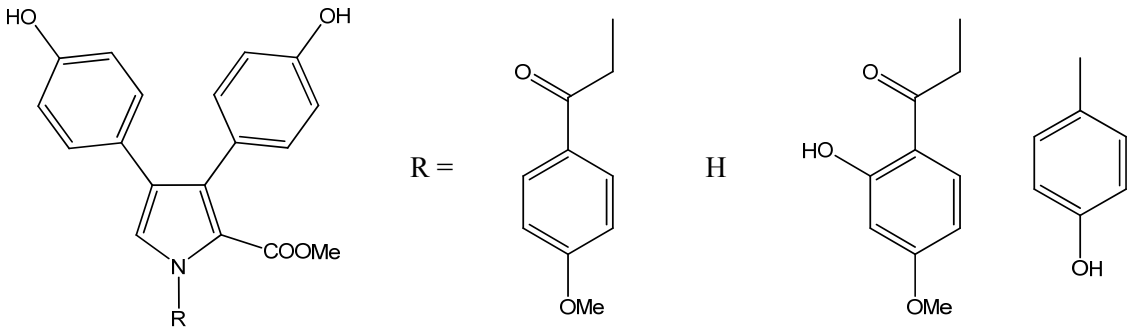



**Figure 2**

### 1.3.2 Biological Activities

Lamellarins exhibit a wide range of interesting and significant biological activity, including cytotoxicity and antitumor activity, multidrug resistance (MDR), HIV-1 integrase inhibition, antibiotic activity, human aldose reductase (h-ALR2) inhibition, cell division inhibition, immunomodulatory activity, antioxidant activity and feeding deterrence [3].

Most of Lamellarins have been found to be cytotoxic to a wide range of cancer cell lines[18]. Among them Lamellarin D has potent cytotoxic activity against various tumor cells, especially human prostate cancer cells ( DU-145, LNCaP) and leukemia cells (K562, P388) [18]. The study shows that Lamellarin D is a potent inhibitor of DNA topoisomerase I, with cytotoxic action that is fully maintained in multidrug resistance cell lines[20]. Lamellarin O also proved to be an effective cytotoxic agent for leukemia and lymphoma, inhibiting (L1210) lymphocytic leukemia DNA synthesis with less effect on RNA and protein synthesis[21].

Some Lamellarins were found to be nontoxic inhibitors of multidrug resistance (MDR) in various cancer cell lines, which may improve the effectiveness of a wide variety of anticancer drugs. Quesada and his co-workers[22],[23] have demonstrated that Lamellarins K and I not only exhibit potent cytotoxic activity in vitro against MDR tumor cell lines but also reverse MDR at noncytotoxic concentrations by inhibiting the P-glycoprotein-mediated drug efflux at nontoxic doses.

Lamellarins with dihydroisoquinoline framework **(1)**, which bear a sulfate group, have shown impressive activity as inhibitors of the HIV-1 integrase enzyme (a promising target for the treatment of acquired immune deficiency syndrome “AIDS”). Faulkner and coworkers reported that Lamellarin α-20 sulfate is a selective inhibitor of HIV-1 integrase both in vitro and in vivo [15],[24].

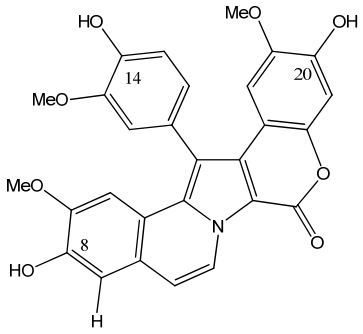
### 1.3.3 Structure-Activity Relationship

Structure-activity relationship (SAR) is a science that explains the source of activity and identifies the pharmacophore in many biologically active compounds. Thus it eventually leads to the design of new molecules that can mimic nature and potentially provide drugs for the treatment of diseases.

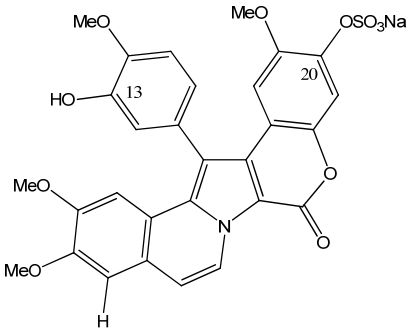
The SAR study of Lamellarin D [25] has revealed that double bond at C5-C6 is an essential element for the antiatopoisomerase I activity. Furthermore the hydroxyl groups at C8, C14, and C20 were proved to be important structural requirements for cytotoxic activity and DNA-topoisomerase I inhibition.

The aforementioned conclusions were confirmed by synthesizing different derivatives of Lamellarins D **(4)** and testing their biological activities. The importance of the hydroxyl group in specific positions was confirmed by acylation of these groups with various carboxylic acids. This considerably reduced the potency, whereas the amino acids derivatives, which preserved the hydrogen bonding capacity at these sites, afforded potent compounds [25],[26] .

An SAR study was also performed on Lamellarin α20-sulfate **(5)** [27]. It demonstrated that the presence of sulfate on the periphery of the compound can greatly influence selectivity in HIV-1 integrase inhibition.




**4**




**5**

# CHAPTER 2

## Synthetic routes to Lamellarins and related natural products

The Lamellarins and related pyrrole-derived alkaloids have attracted a lot of interest among synthesis researchers, due to their interesting biological activity coupled with the scarcity of these materials among natural sources.

In order to synthesize such compounds, several synthetic approaches have been tried. These can be classified into two main categories based on the construction of the pyrrole ring. In the first approach, the formation of pyrrole core is accessed at a later stage of synthesis, and the highly functionalized precursors into the pyrrole core of the targets were assembled by means of intramolecular cycloaddition[28],[29],[30],[31],[32], azadiene Diels-Alder cycloaddition[33], oxidative dimerization[34],[35], or double-barreled Heck cyclization[36] (Figure 3).

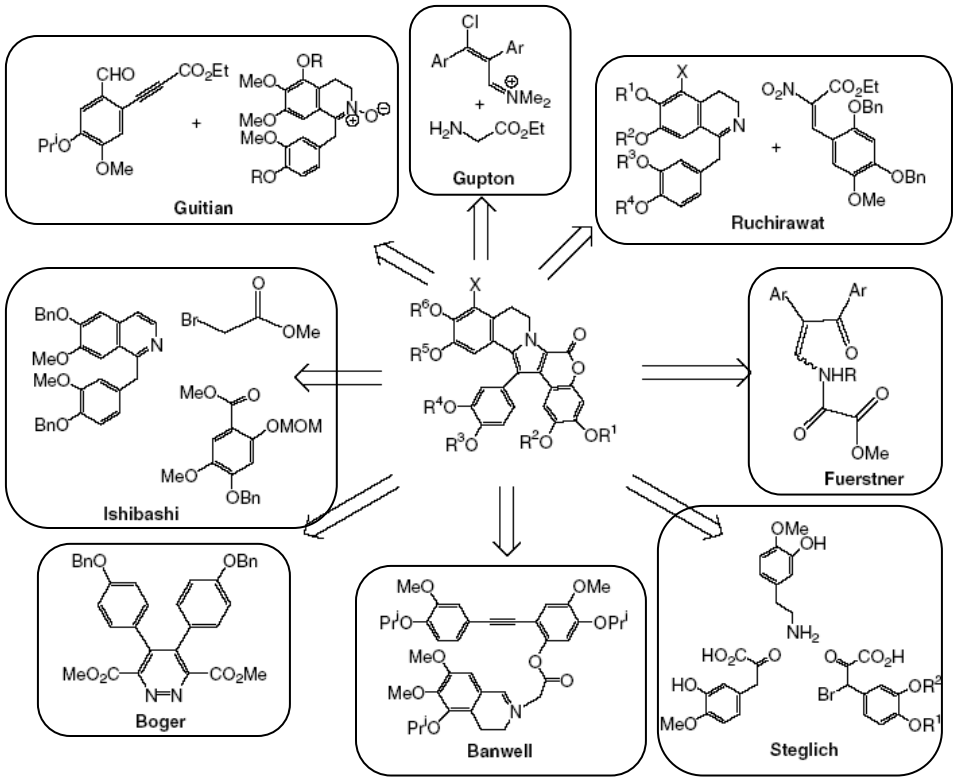



**Figure 3**

In the second approach, an intact pyrrole core is elaborated, but this process faces difficulties in functionalizing the pyrrole ring in the regiocontrolled fashion. It is therefore less explored (Figure 4).

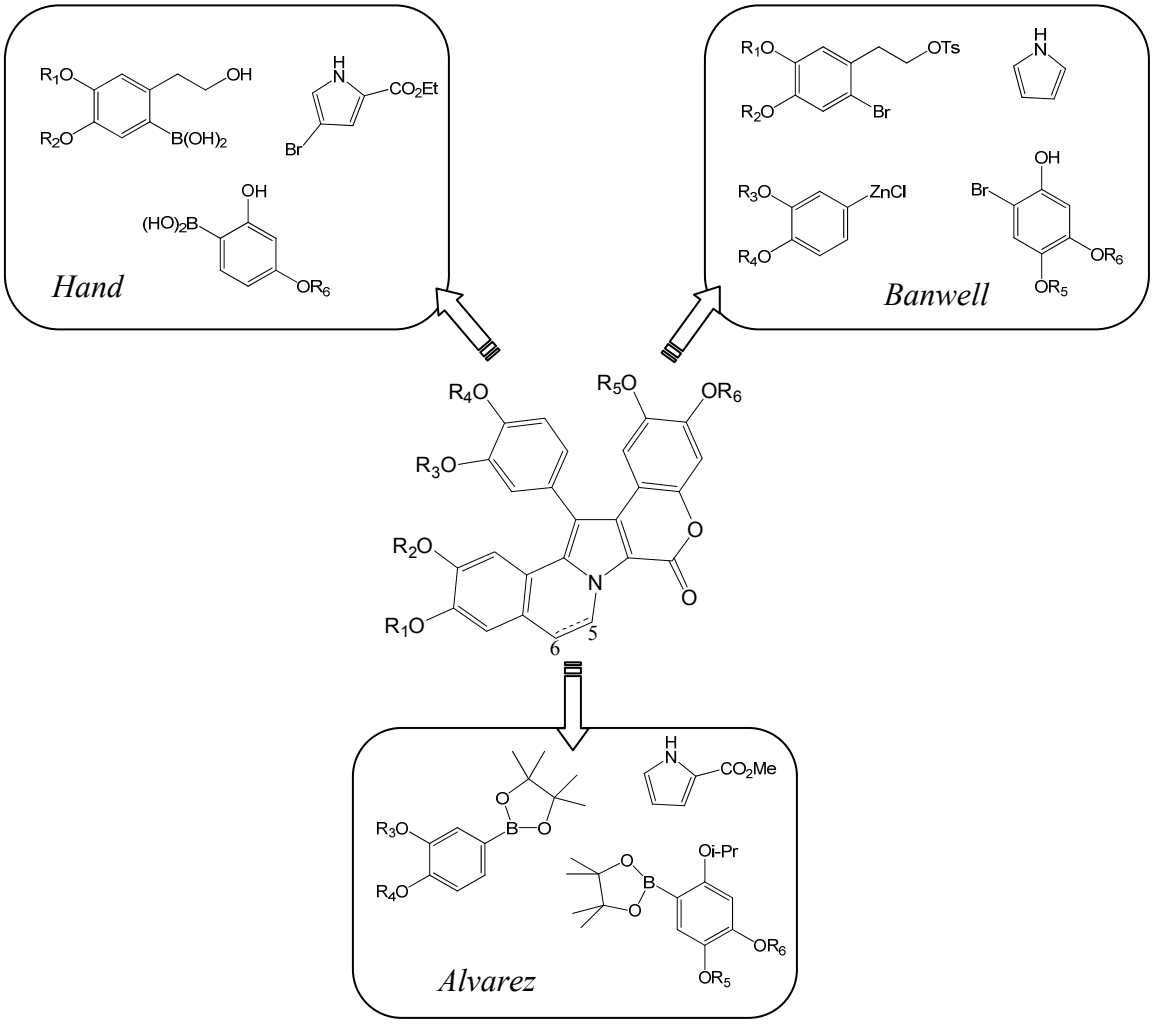



**Figure 4**

## 2.1 Late stage pyrrole formation approaches

### 2.1.1 Biomimetic synthesis (Steglich approach)

The first biomimetic synthesis of Lamellarins was accomplished by Steglich and his co-workers in 1997[37]. They synthesized Lamellarin G trimethyl ether from arylpyruvic acids by two consecutive oxidative reactions. The first reaction included the oxidative coupling of two arylpyruvic acids, followed by condensation with a suitable 2-arylethylamine. The second oxidative reaction was between an aryl ring and carboxylic acid to give a lactone ring, which was in turn transformed to the isoquinoline framework by intramolecular Heck-type reaction leading to the final product (Figure 5).

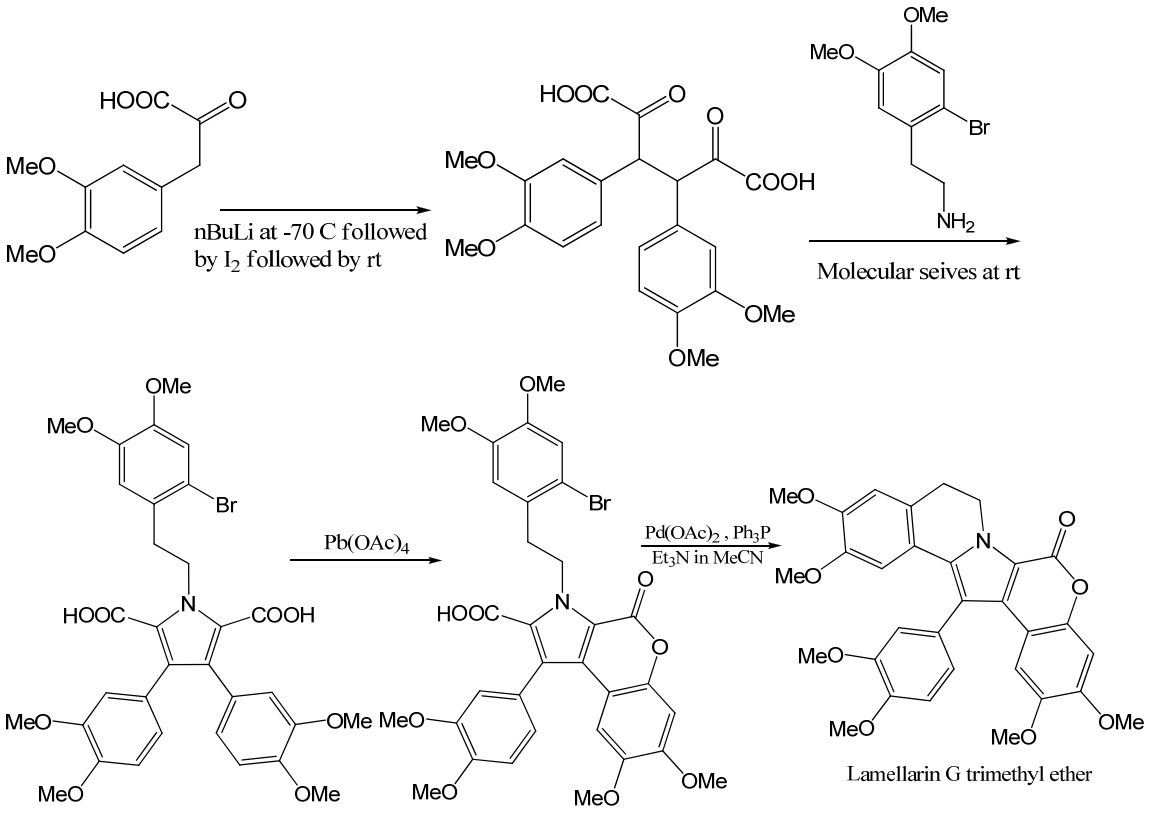



**Figure 5**

To access a nonsymmetrical Lamellarin molecule such as Lamellarin L, two different arylpyruvic acid ester units were employed [38]. Additional precautions were applied to attain the desired structure, including (a) the prior formation of one of the arylpyruvic acid units as the α-halo compound, and (b) the use of two different esters of arylpyruvic acid (Figure 6).

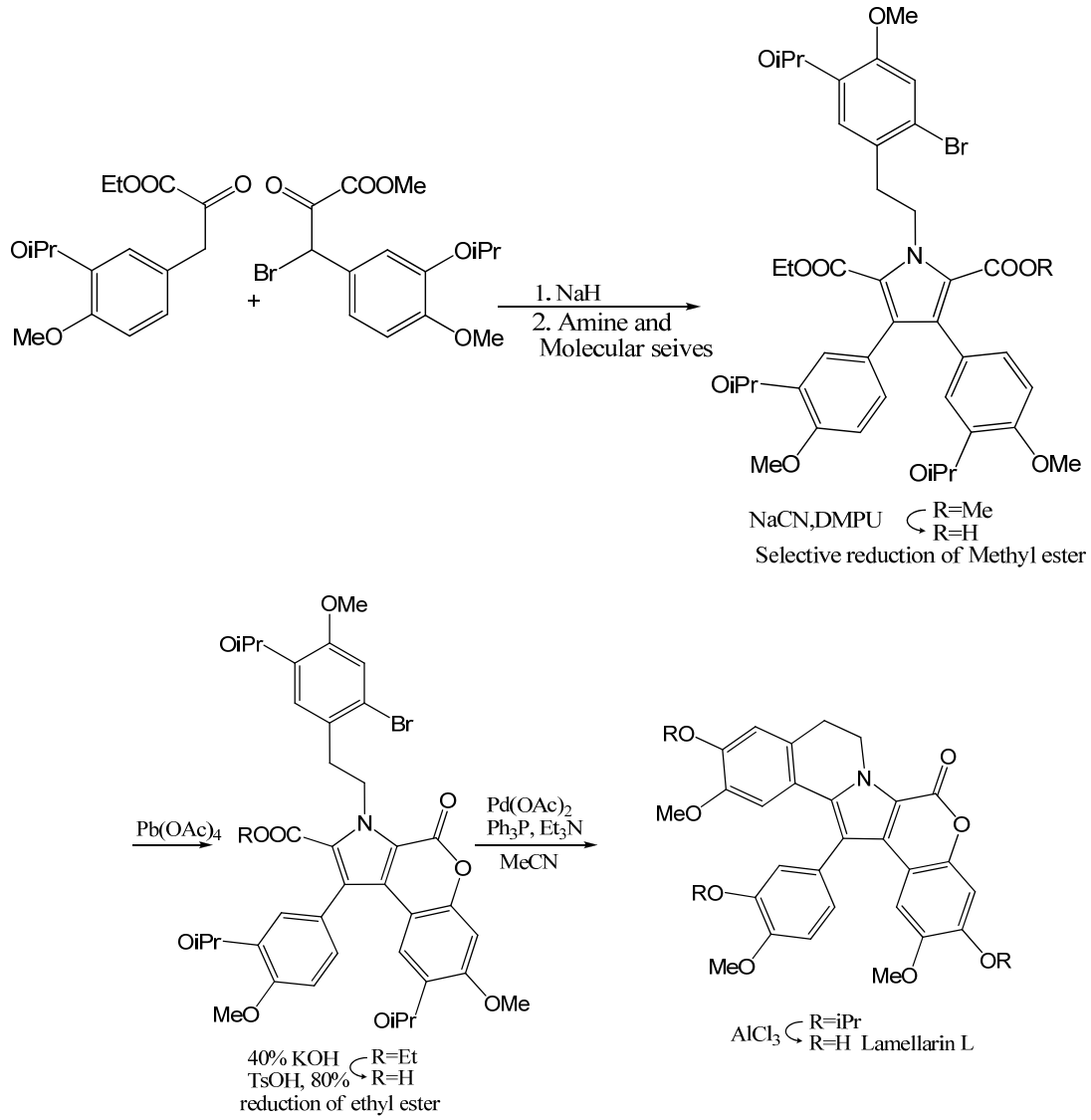



**Figure 6**

Ester differentiation was accomplished by using methyl and ethyl esters, which can be cleaved selectively by using different reagents.

## 2.1.2 Hinsberg-type pyrrole synthesis (Iwao approach)

Ishibashi and Iwao have reported a novel synthetic method for the preparation of 3,4-diarylpyrrole unit to accomplish the total synthesis of Lamellarin G trimethyl ether [38], D, L and Lamellarin N[40]. The proposed method was developed by using Hinsberg-type pyrrole synthesis (Figure 7), followed by a palladium-catalyzed Suzuki cross-coupling reaction of the 3,4-dihydroxypyrrole bis-triflate derivatives as key reactions.

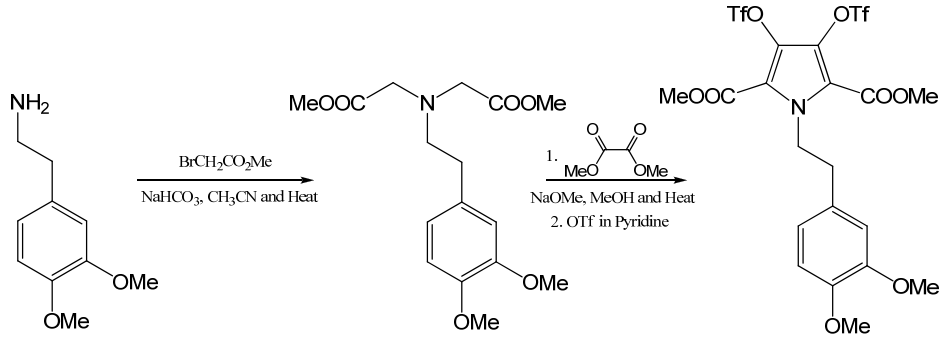



**Figure 7**

The reported method was flexible enough to be applied for the synthesis of different Lamellarins, since the preparation of bis-triflates intermediate allows the coupling of different substituted phenylboronic acids. An interesting feature of this reaction is that it can be used for the preparation of nonsymmetrical Lamellarins by controlling the coupling conditions to get a mono-arylated intermediate, which in turn can be further coupled with a different substituted-arylboronic acid (Figure 8).

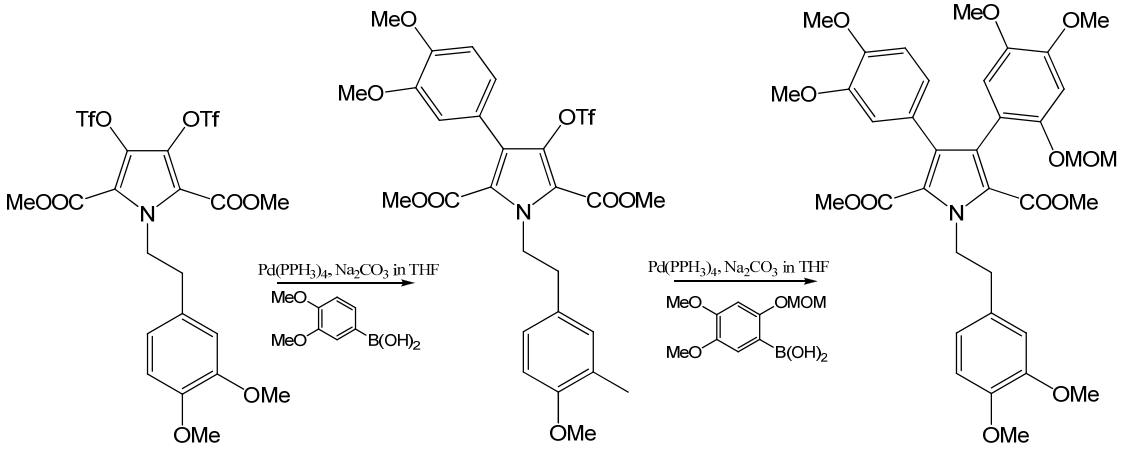



**Figure 8**

## 2.1.3 Vinylogous Iminium Chemistry (Gupton approach)

Vinylogous iminium ion chemistry was also utilized for the synthesis of Lamellarins and their related natural products[41],[42]. Total syntheses of Lamellarin O dimethyl ether and lukianol A have been accomplished in a regiocontrolled fashion. In this way, the key intermediate (Furstner intermediate) was accessed in just three steps: (a) conversion of appropriate ketone to vinylogous amide; (b) transformation of this amide into chloropropeniminim salt; (c) condensation with glycine methyl ester hydrochloride under basic conditions[43] (Figure 9).

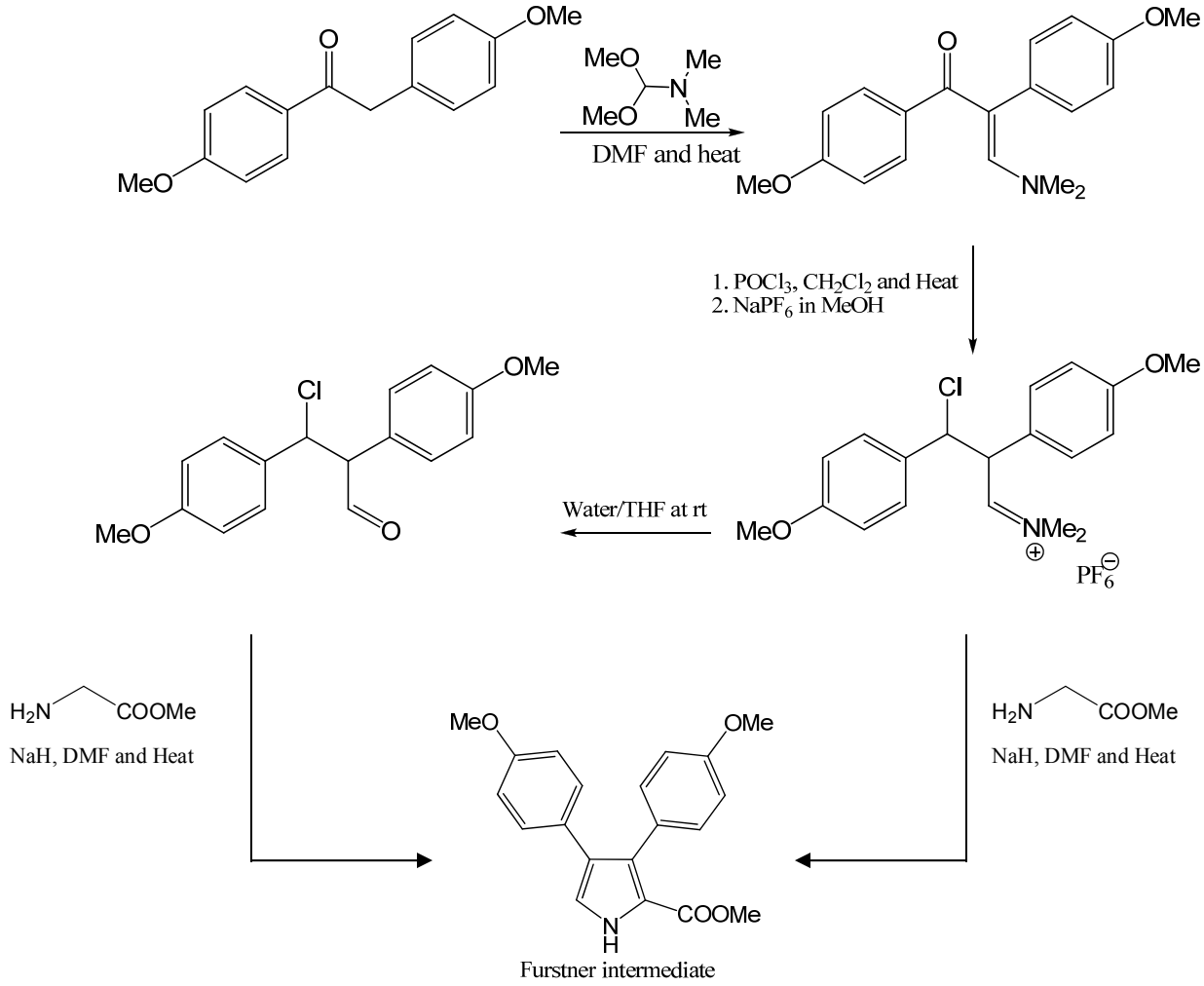



**Figure 9**

### 2.1.4 Cycloaddition of Methylisocyanoacetate with α,β-unsaturated nitriles (Bullington approach)

The Bullington research group in 2002 [43] developed a very efficient and concise synthesis of Furstner intermediate by using a 2+3 cycloaddition reaction of α,β-unsaturated nitriles with methyl isocyanoacetate (Figure 10).

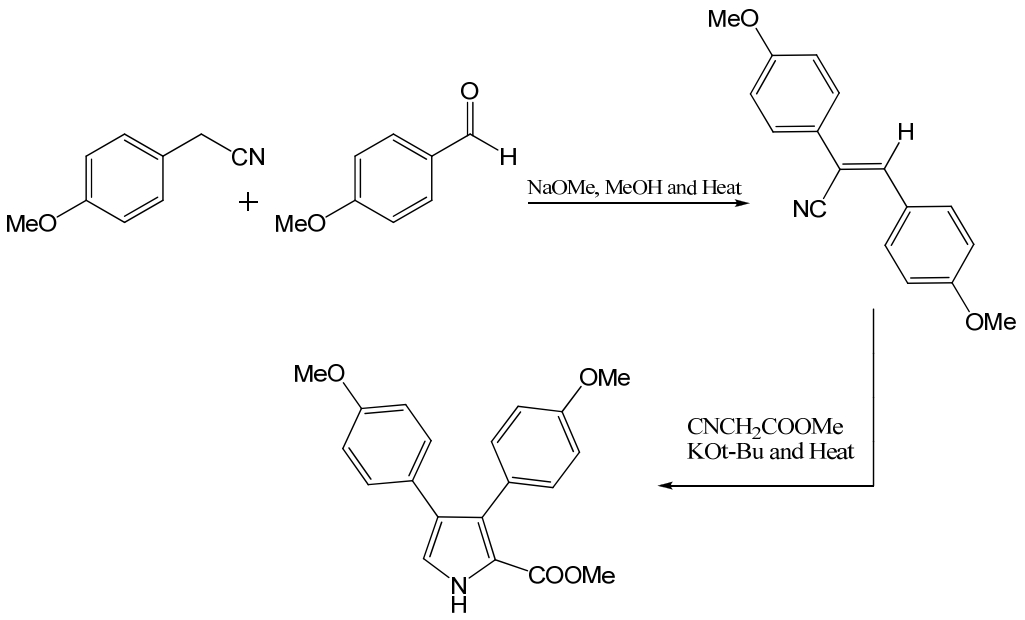



**Figure 10**

The ease of preparation of α,β-unsaturated nitriles, by condensation of benzyl nitriles with aromatic aldehydes under basic conditions, allowed the incorporation of various aromatic groups into the pyrrole ring by reacting electron-deficient α,β-unsaturated nitriles with methyl isocyanoacetate, which in turn has permitted the control of regiochemistry of the final product.

Using this method, Bullington and his colleagues reported the total synthesis of Ningalin B in only three steps giving 51% overall yield.

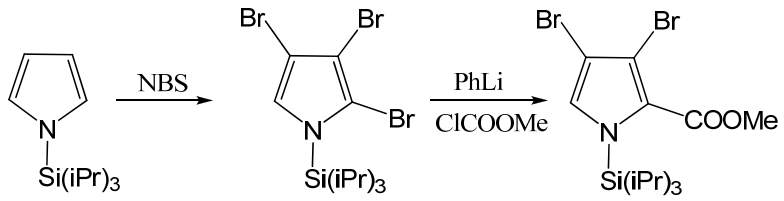
## 2.2 Functionalization of Pyrrole ring

The second methodology to synthesize the Lamellarins framework is to begin with an intact pyrrole ring, and then to functionalize it. The challenge here is to establish a reliable method for regioselective functionalization of the pyrrole. Most attempts in this strategy include the application of cross-coupling chemistry on halogenated pyrrole.

### 2.2.1 Double cross-couplings (Banwell / Iwao approaches)

In the cross-coupling approach[45] the key synthesis step involves the Stille, Suzuki or Negishi cross-coupling reaction of readily available pyrrole 2-carboxylic acid derivatives with the appropriate aryl stannane, boronic acid or iodide, catalyzed by palladium metal.

The dibromo pyrrole 2-carboxylic acid derivative was easily accessed from pyrrole triisopropylsilyl derivative by reacting it with N-bromosuccinimide (NBS) in THF [45].




Lithium exchange of the tribromo derivative with PhLi, followed by quenching with acid chloride, afforded the key intermediate, which was coupled with the appropriate aryl stannane or aryl boronic acid to obtain a symmetrically arylated Furstner intermediate (Figure 11).

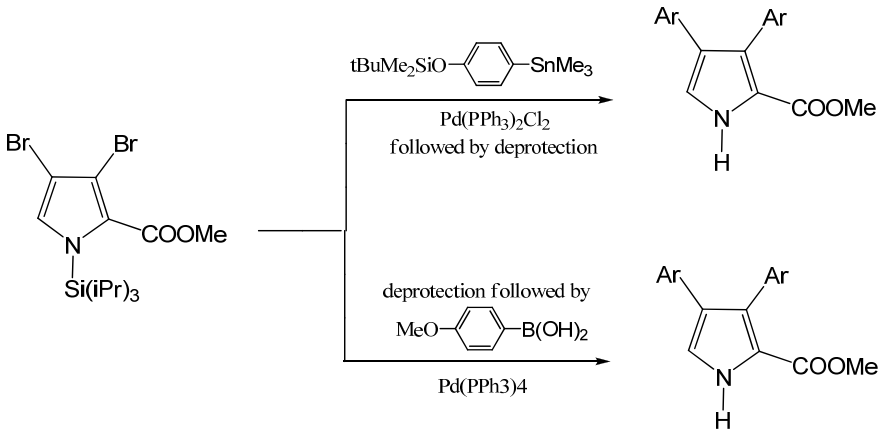



**Figure 11**

The limitation of this strategy was evident when it was applied to synthesize differentially the di-arylated pyrroles, since the reported cross-coupling reactions could not be controlled to get mono-arylated pyrroles. This drawback was overcome by regioselective lithiation, followed by transmetallation and the Negishi cross-coupling reaction with aryl iodide (Figure 12).

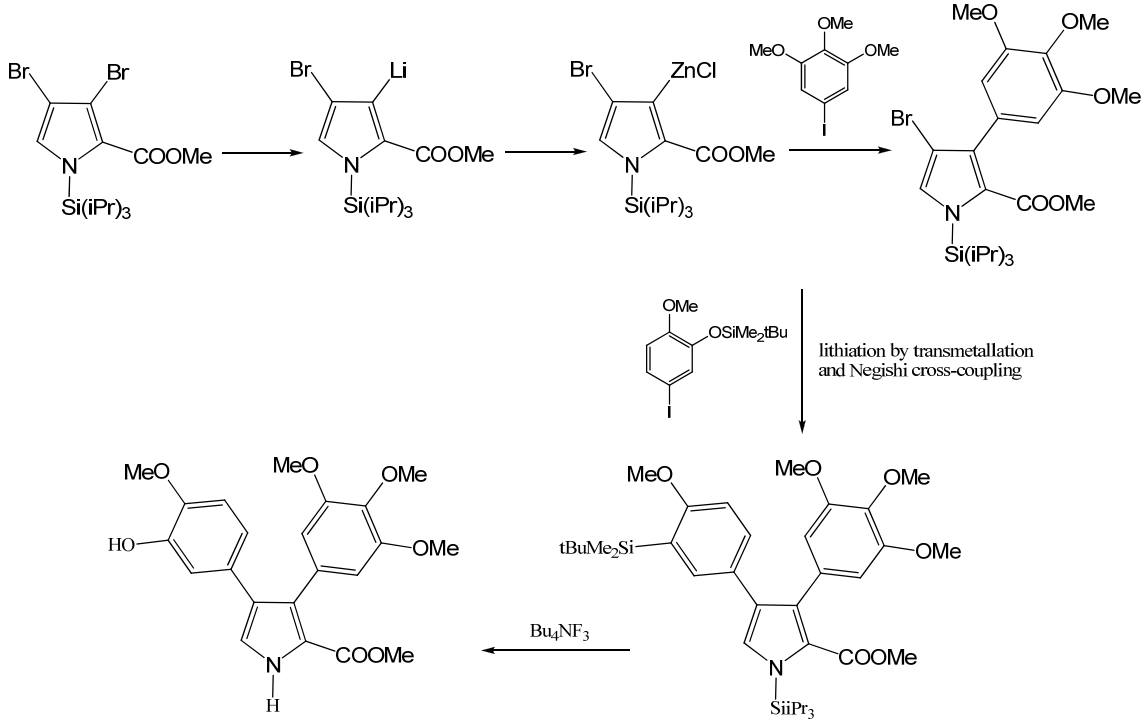



**Figure 12**

An interesting modification of the previous strategy utilized the attachment of *p*-toluenesulfonyl group on nitrogen, being an activated and directing group, to prepare 3,4-dibromo-N-(*p*-toluenesulfonyl) pyrrole via direct bromination[46] (Figure 13).

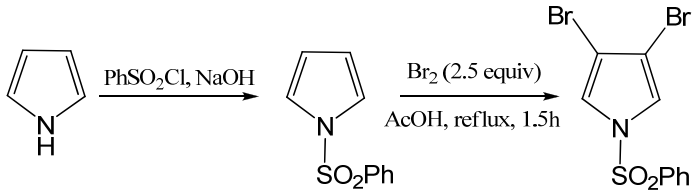



**Figure 13**

The results indicated that N-benzenesulfonyl-3,4-dibromopyrrole is far superior to 3,4-dibromo-N-triisopropylsilylpyrrole as a substrate, at least in Suzukie-Miyaura cross-coupling. The electron-withdrawing N-benzenesulfonyl group facilitates the initial oxidative insertion of C-Br bonds to Pd(0) and, as a consequence, it promotes an overall cross-coupling reaction cleanly to get the mono-arylated pyrroles with high regioselectivity (Figure 14).

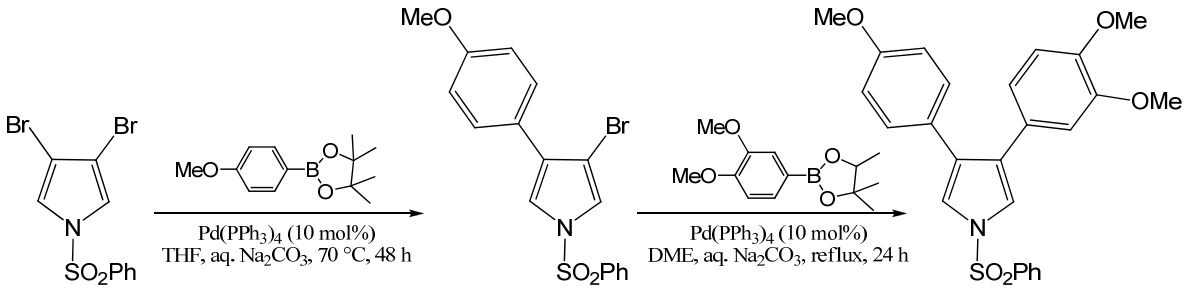



**Figure 14**

## 2.2.2 Heck/Suzuki couplings (Alvarez approach)

In 2005, Alvarez and colleagues adopted the Heck coupling reaction as a key reaction to synthesize a Lamellarin skeleton[47]. Alkylation of pyrrole ester with tosylate derivatives, followed by the Heck reaction, produced a dihydroisoquinoline pyrrole derivative, which could be monobrominated at pyrrole C-4 position or dibrominated at C-4 and C-5 positions, followed by coupling with aryl boronic acids (Figure 15).

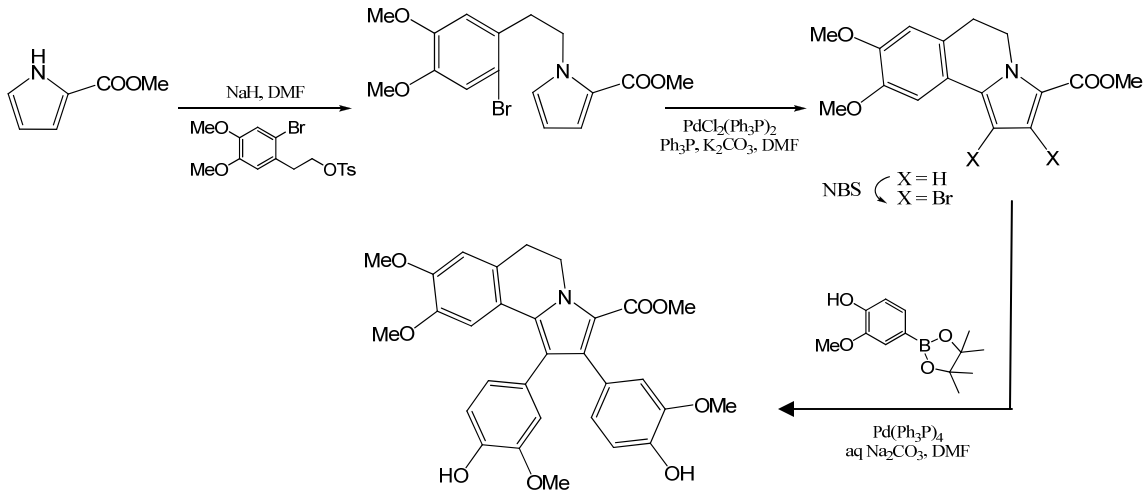



**Figure 15**

The reported synthesis was the most versatile and flexible way to prepare different substituted Lamellarins from the intact pyrrole core.

# CHAPTER 3

# Objectives and work plan

## 3.1 Importance of the proposed research

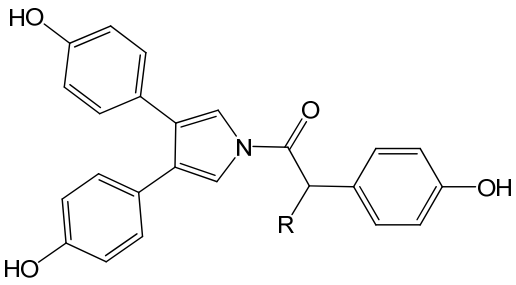
Huge efforts have been made in recent decades to discover potent drugs for cancer treatment. Biochemists, associated with medicinal and synthetic chemists, have tried to exploit the diversity of natural products available from marine sources.

The objective of this study was to achieve the first total synthesis of two different marine natural products, which were isolated from sponges and have proved to be potential anticancer agents. The two different alkaloids, Neolamellarin and Aplysamine 6, were chosen due to their potent bioactivity as inhibitors of different anticancer targets.

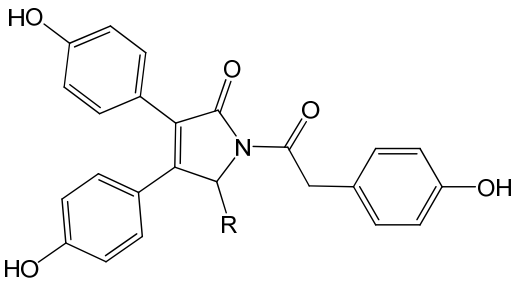
### 3.1.1 Neolamellarin as HIF-1 inhibitor

In search of potent and selective small-molecule HIF-1 inhibitors taking advantage of the vast biochemical diversity afforded by natural products, the NCl Open Repository of marine invertebrates and algae lipid extracts. The biological activity was evaluated for HIF-1 inhibitory activity in a T47D human breast tumor cell-based reported assay. The crude extract of the sponge Dendrilla nigra (Aplysillidate) inhibited the hypoxia-induced HIF-1 activation (81% inhibition at 5 $\mu g.ml^{-1}$) [47]. Bioassay-guided chromatographic separation of the *D.nigra* extract yielded four new Lamellarin-like phenolic pyrroles neolamellarins: neolamellarin A **(6)**, neolamellarin B **(7)**, 5-hydroxyneolamellarin C **(8)**, and 7-hydroxyneolamellarin D **(9)**, which bore structural features similar to lamellarin O **(10)** [47], originally isolated from the Australian sponge *D. cacto s* [10] ( Figure 16).

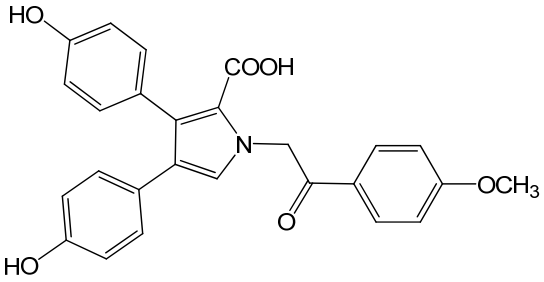
The effects of **(6-9)** on HIF-1 activity in a T47D cell-based reporter assay revealed that compound **(9)**, being the most active, inhibited the hypoxia (1% $O_2$)-induced HIF-1 activation with an $IC_{50}$ value of 1.9 μM. At the highest concentration (10 μM), the percentage inhibition exerted by **(6-9)** on HIF-1 activation was 26% **(6)**, 23% **(7)**, 22% **(8)**, and 98% **(9)**, respectively [47]. Moreover compound **(9)** decreased the induction of secreted vascular endothelial growth factor (VEGF) protein, one of the most studied HIF-1 regulated target genes and a key factor that promotes tumor angiogenesis, in T47D cells by hypoxia (1% $O_2$) and in 1,10-phenanthroline (10 μM) by 87% and 33%, respectively[35].




(6) R=H , (9) R=OH




(7) R=H , (8) R=OH




(10)

**Figure 16**

The limited quantity of **(9)** hindered the studies from elaborating the mechanism of action studies and further investigation of its potential as a molecular-targeted antitumor agent. There was a growing need to develop a synthetic way to access these targets, in order to conduct more extensive pharmacological studies of them.

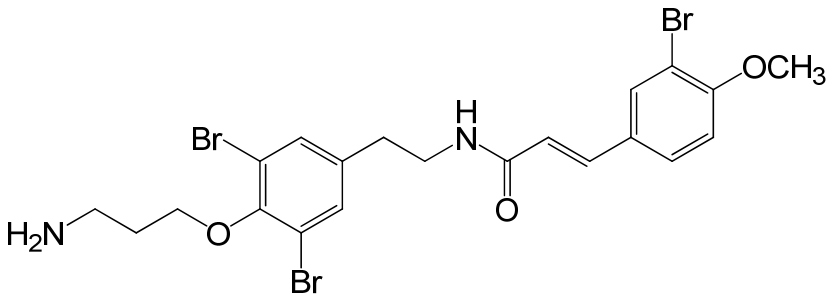
### 3.1.2 Aplysamine 6 as inhibitor of (Icmt) anticancer target

The C-terminal isoprenylcysteine motif of the CaaX proteins targets a variety of eukaryotic proteins to a series of post-translational modifications which are important for their localization and function.[49],[50] To initiate the processing, the protein farnesyltransferase, or the protein geranyl geranyltranferase type I, catalyze the covalent attachment of a 15-carbon farnesyl or a 20-carbon geranylgeranyl lipid to the cysteine of the CaaX motif.[51] After prenylation and followed by removal of three C-terminal amino acids, the C-terminal isoprenylcysteine is methylated by isoprenylcysteine carboxy methyltransferase (Icmt).[52]

Proteins that terminate in a CaaX motif regulate a number of important pathways in oncogenesis. The most thoroughly studied example is the central role of the Ras family of proteins in the growth factor activation of the MAP kinase signaling cascade, In addition, many cancers contain alterations upstream of Ras, and the resultant hyperactivation of Ras is thought to contribute to tumorigenesis in these cancers as well [52]. Recent studies using the genetic disruption of Icmt revealed that Ras proteins are significantly mislocalized, and tumorigenesis is markedly impaired, in cells that lack Icmt.[53]

With emerging evidence for the importance of Icmt-catalyzed CaaX protein methylation in oncogenesis, there is a growing need for specific pharmacological agents to target this process. The development of Icmt inhibitors is a new way to find anticancer drugs.[54] In order to discover new Icmt inhibitors, bioassay-directed purification of extracts of the sponge *Pseudoceratina* sp (Pseudoceratinidae) produced a new bromotyrosine derivative, aplysamine 6 **(11)**. [54] Aplysamine 6 contains one bromotyrosine unit and one bromomethoxycinnamoyl unit. It shows inhibition of Icmt with an $IC_{50}$ of 14 μM (assay performed in duplicate on four independent days), and it is considered a new addition to the small list of inhibitors of Icmt. [54].

We aimed to accomplish the first total synthesis of Aplysamine 6 **(11)**.




**11**

## 3.2 Objectives

We aimed to adopt a facile synthetic approach to synthesize Neolamellarins A **(6)** and Aplysamine 6 **(11)** by utilizing very common starting materials available in-house. Our objectives were:

1 – To study different approaches towards the syntheses of 3,4-diaryl pyrrole by utilizing a pyrrole ring formation strategy.

2 – To optimize the demanding step of N-acylation of the pyrrole ring, in order to access the high functionalize intermediates and to accomplish the total synthesis of Neolamellarin A.

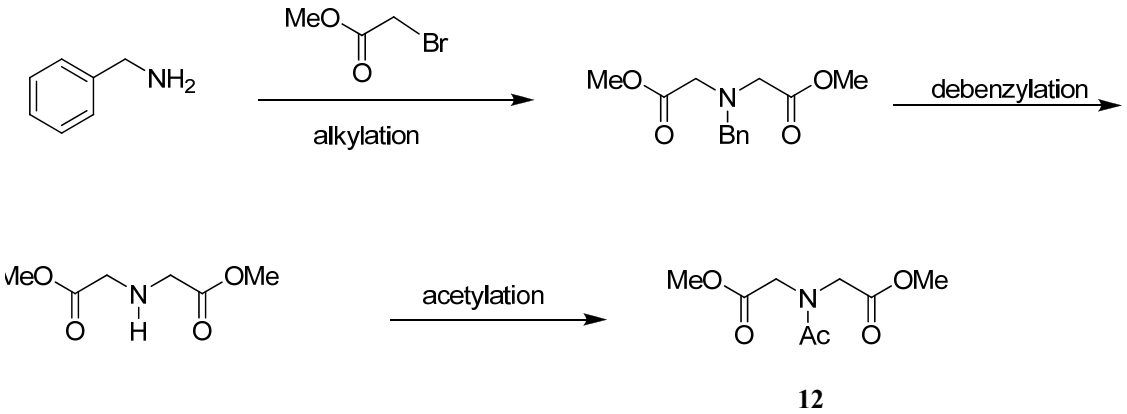
3 – To develop a cost-effective and easy-to-exercise synthetic pathway to achieve the first total synthesis of Aplysamine 6.

## 3.3 Methodology and the work plan

### 3.3.1 Synthesis of Neolamellarin A

To synthesize neolamellarin A the key step of our strategy centered on the base-mediated cyclization, followed by three steps (decarboxylation, alkylation and deprotection) to attain the target compounds.

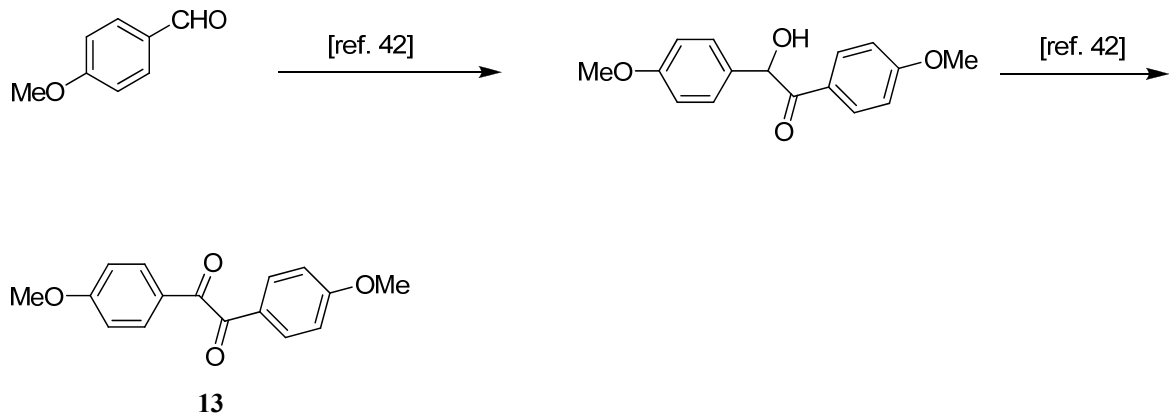
The first part of this project was to prepare the key intermediate **(12)**, which will be synthesized by three operations (alkylation, removal of benzyl group, and subsequent acetylation) as described in Figure 17.




**12**

**Figure 17**

Parallel to that, another key segment, the dione **(13)**, was required to accomplish the target compounds, and it was to be synthesized according to the literature [54](Figure 18).




**13**

**Figure 18**

Later, the base-induced coupling of intermediates **(12)** and **(13)** allowed access to the pyrrole core advanced intermediate **(19)**, which was converted to an intermediate **(20)** by hydrolysis and decarboxylation. The advanced intermediate **(20)** would in turn be subjected to amidation with intermediate **(41)** to attain **(44)**, followed by demethylation to get the final target compound (Figure 19).

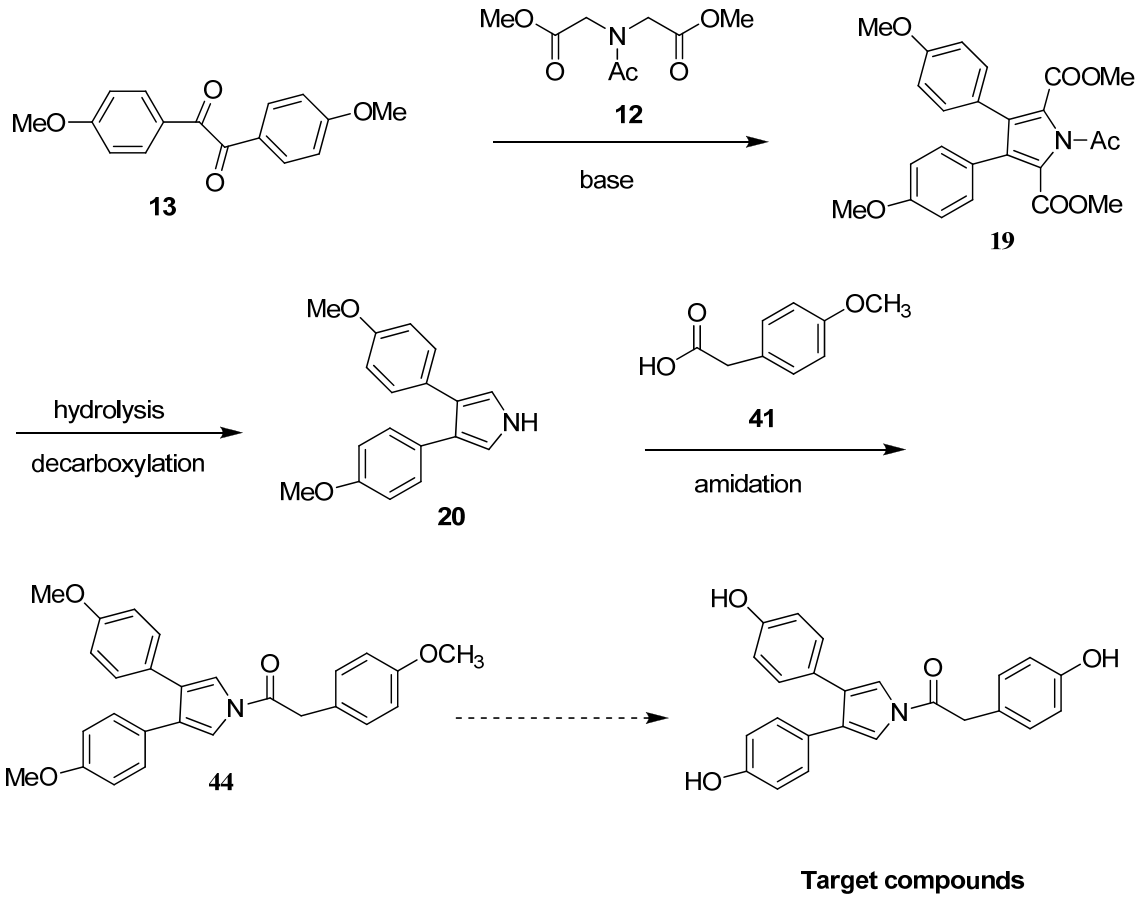
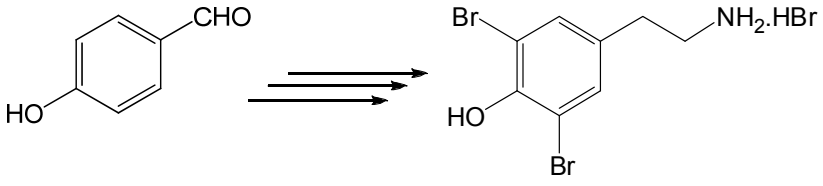



**Figure 19**

### 3.3.2 Synthesis of Aplysamine 6

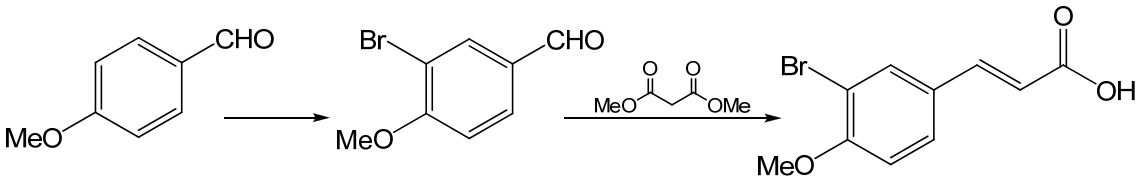
Aplysamine 6 will be prepared from highly functionalized key intermediates, amine **(56)** and acid **(46).**

Intermediate **(56)** in turn will be prepared from 4-hydroxybenzaldehyde **(48)** by bromination, followed by the Henry reaction and reduction of the double bond.



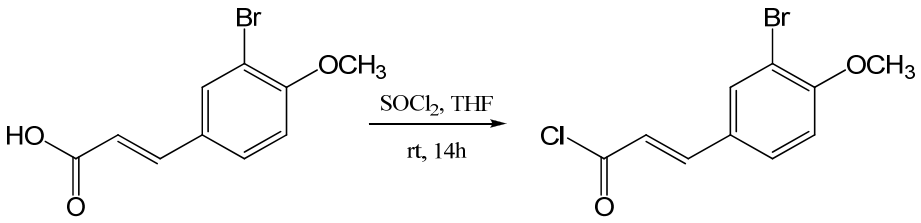

**48** **56**

The acid intermediate **(46)** will be prepared by the conversion of *p*-anisaldehyde **(21)** to the known bromobenzaldehyde **(47)**, followed by Doebner–Knoevenagel condensation with malonic acid to afford the cinnamic acid **(46).**



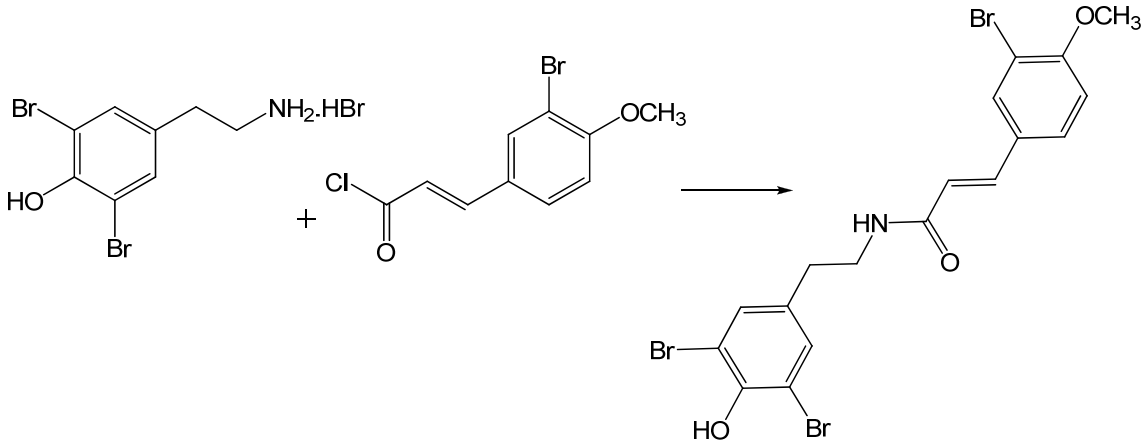

**21** **47** **46**

Once the cinnamic acid **(46)** is in hand, it will be converted into an acid chloride derivative **(61)**, which will be condensed with amine **(56)** to achieve the advanced intermediate **(62)**.

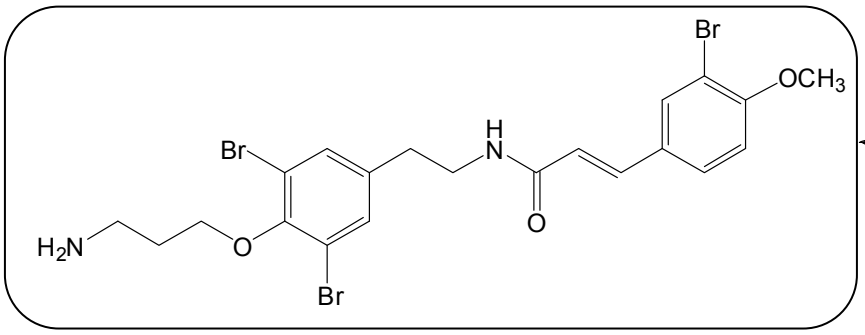



**46** **61**

The advanced intermediate **(62)** will be ultimately advanced to the target compound Aplysamine 6 **(11)**.




**(62)**




Two Steps

**(11)**

# CHAPTER 4

# Results and Discussions

## 4.1 Synthesis of Neolamellarin A

In order to achieve the total synthesis of Neolamellarin A, the pyrrole subunit of the compound was synthesized by adopting different approaches as outlined in (Figure 20).

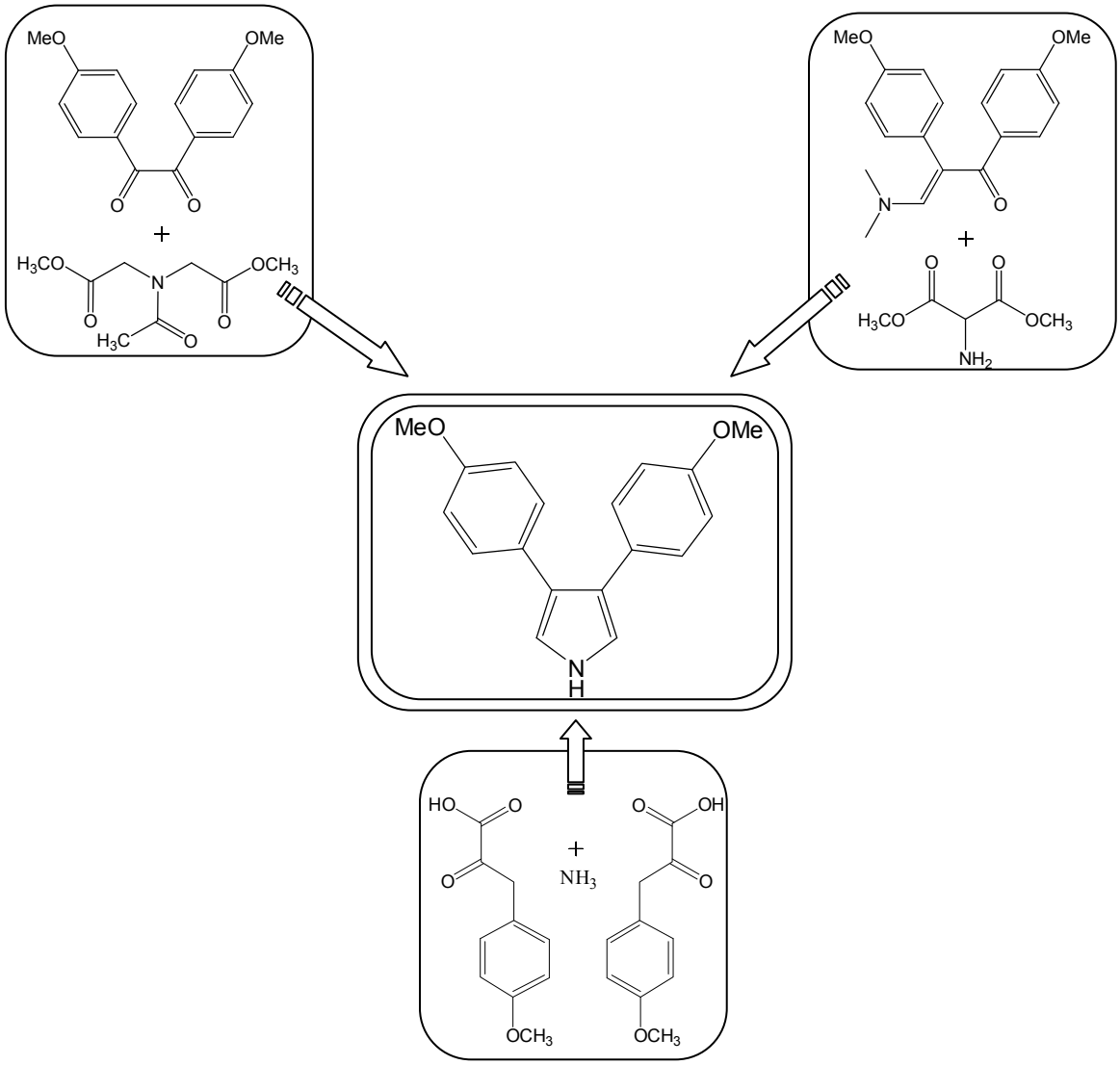
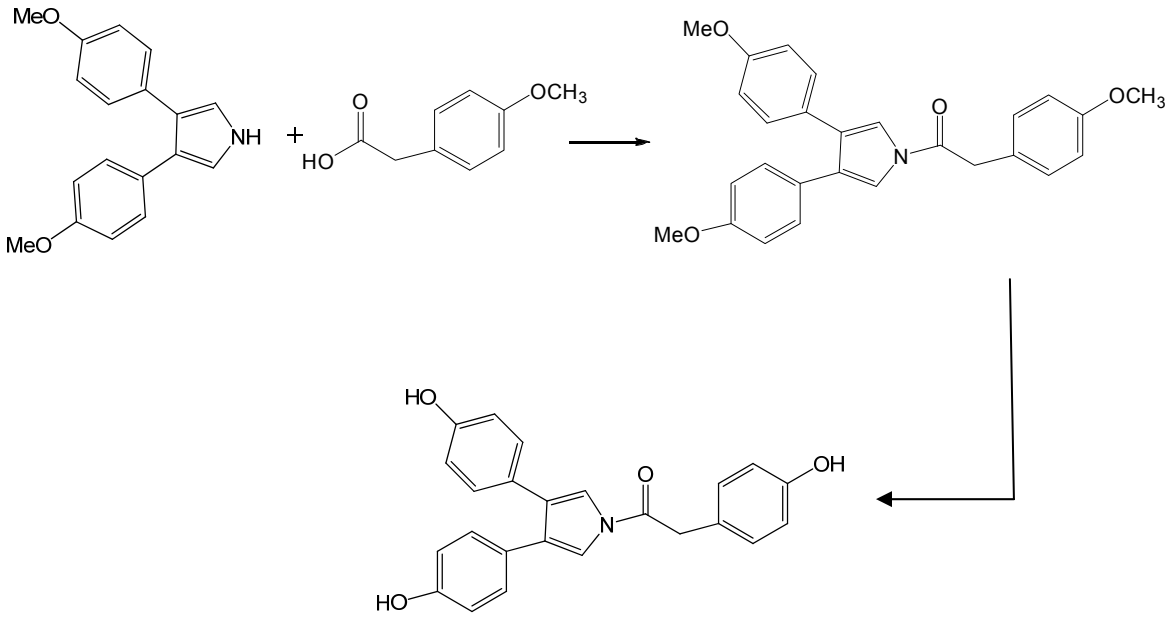



**Figure 20**

The other segment of the compound, in form of acid chloride derivative, was attached to the pyrrole core by means of N-acylation reaction, followed by deprotection to get the final target compound.

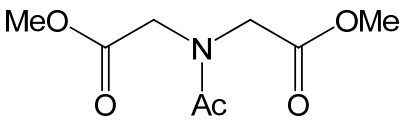



**Target compounds**

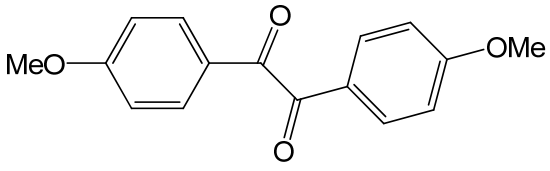
## 4.1.1 Synthesis of 3,4-diaryl pyrrole

### 4.1.1.1 Hinsberg-type pyrrole synthesis

Formation of pyrrole ring, the key step in our synthesis approach, was accessed by Hinsberg-type condensation of the iminodiacetates **(12)** with Dion **(13)**.

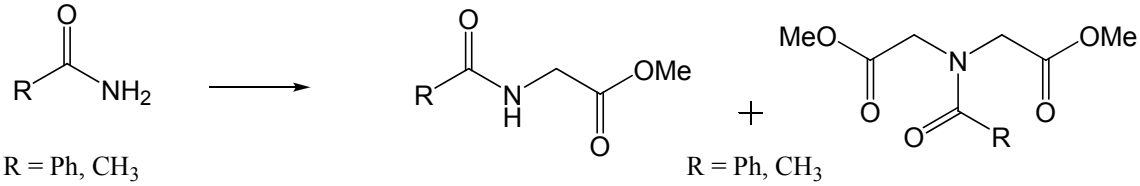



**12**

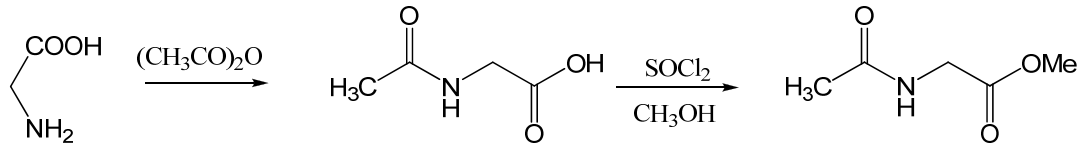



**13**

In order to synthesize intermediate **(12)**, the alkylation of acetamide or benzamide were proved to be unsatisfactory resulting in a mixture of monoalkylated and dialkylated products.



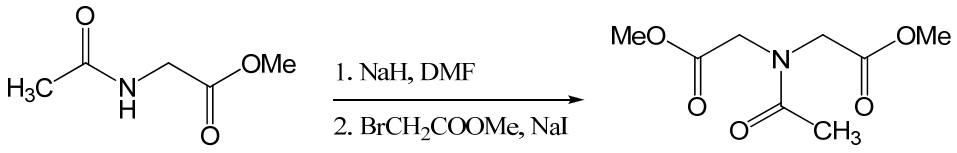

This problem was overcome by developing an alternative procedure as outlined in Scheme 1. Acetylation of glycine **(14)** with acetic anhydride gave the corresponding acetyl-glycine **(15)**, which in turn was esterificated by the action of thionyl chloride in MeOH.




**14** **15** **16**

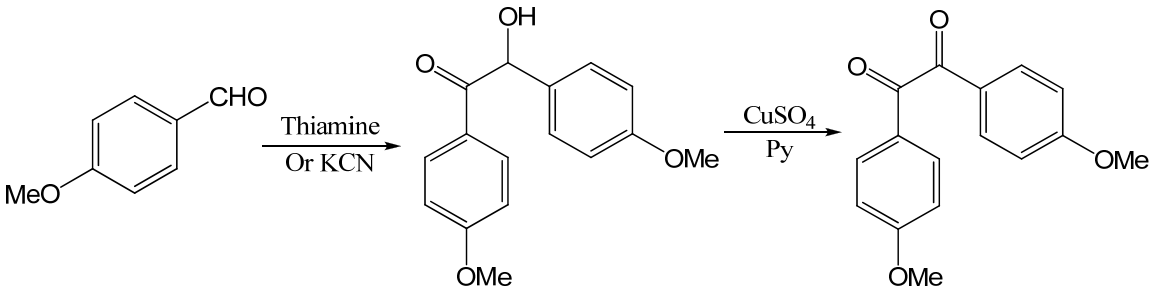
**Scheme 1**

The N-alkylation of the intermediate **(16)** with methyl 2-acetamidoacetate in DMF, by using sodium hydride as a base, produced the desired intermediate **(12)** in an excellent overall yield (90%).



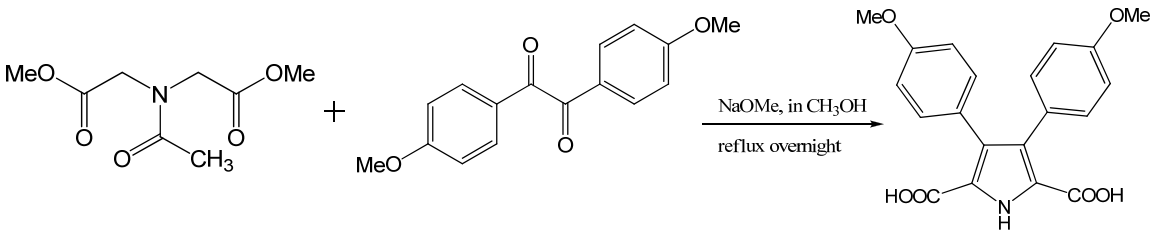

**16** **12**

The other key intermediate, the dione 3,3′-dimethoxybenzyl **(18)**, was prepared by a standard benzoin condensation reaction, using thiamine or potassium cyanide as a catalyst affording 2-hydroxy-1,2-bis(4-methoxyphenyl)ethanone **(17)**, which was oxidized by $CuSO_4$ in pyridine to attain the desired dion **(18)** with a 66% overall yield.



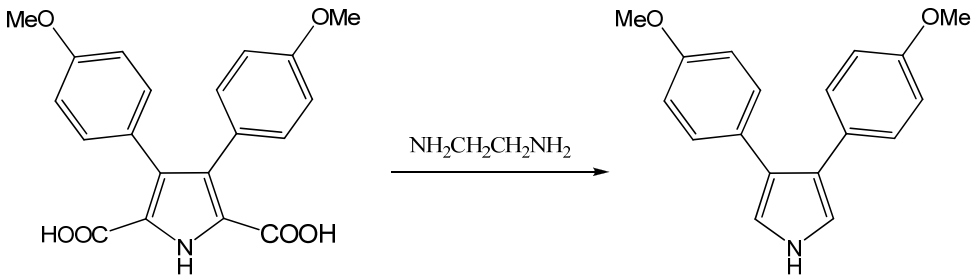

**17** **18**

The base-induced Hinsberg-type condensation of the N-acetyliminodiacetate **(12)** with 3,3′-dimethoxybenzyl **(13)** was attempted by using sodium methoxide as a base in refluxing methanol to get the acid intermediate **(19)**, which was purified by using acid-base extraction, and then recrystallized from methanol-water (1:1) to get the product as a white needle solid.




**19**

Finally, decarboxylation of the acid intermediate **(19)** was attempted by using ethanol amine at elevated temperature according to the literature precedent [56], which was proved to be low-yielding.




**19** **20**

Thus, the overall sequence for synthesizing the pyrrole **(20)** by Hinsberg-type condensation required several reaction steps coupled with low yields. This suggested a need to revise and redesign the strategy to access the pyrrole **(20)**.

The reaction mechanism for the formation of pyrrole intermediate **(20)** is believed to be as below (Figure 21).

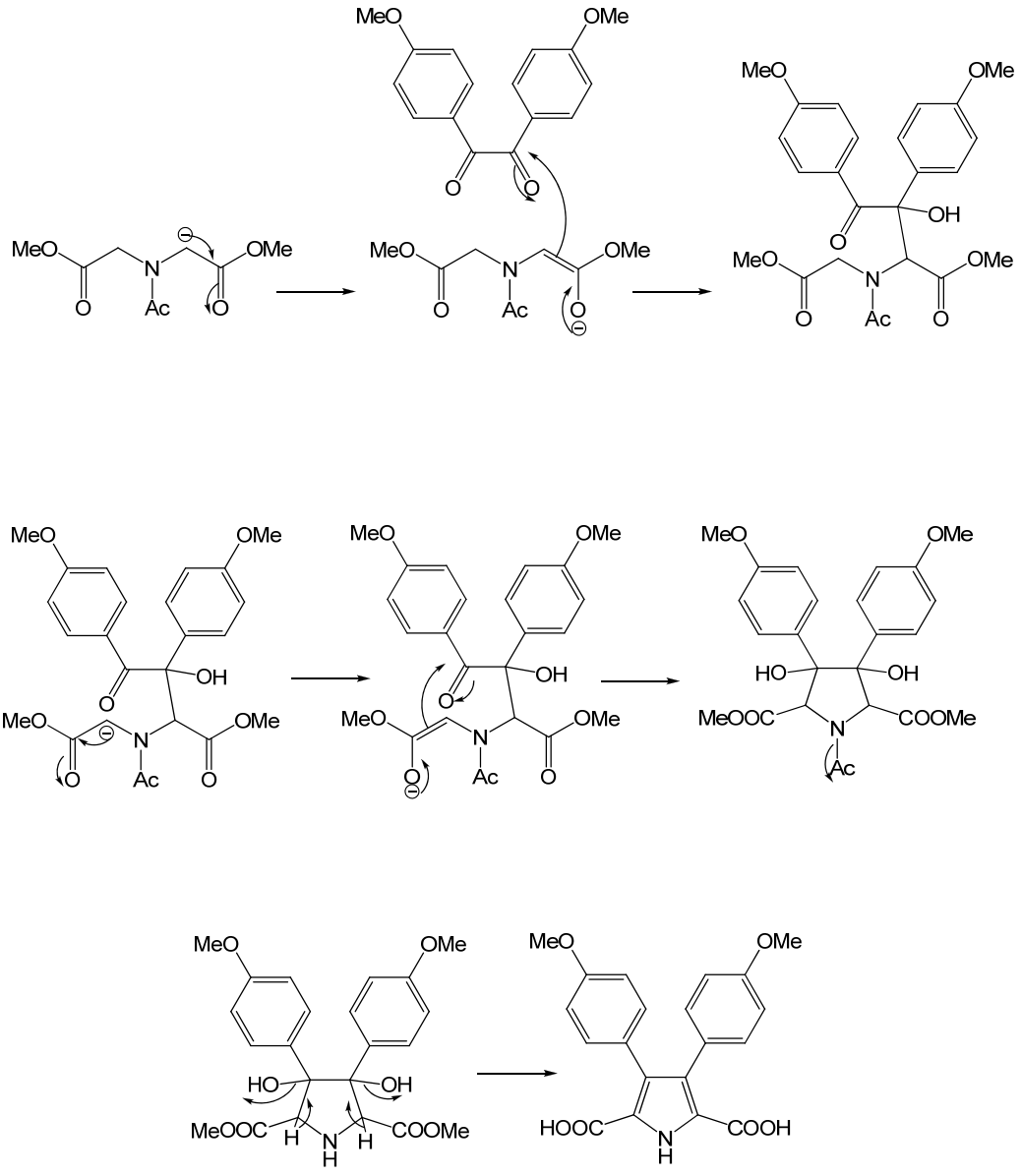
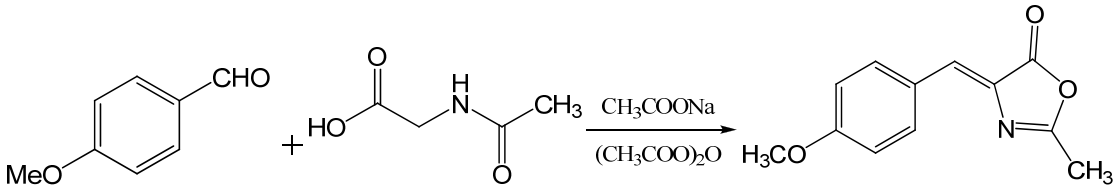


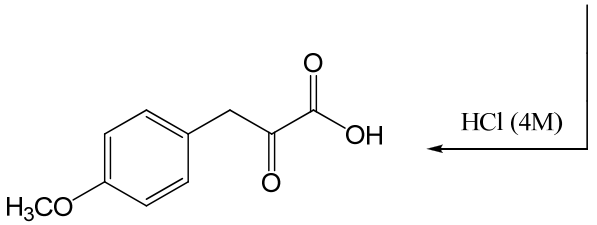

**Figure 21**

### 4.1.1.2 Arylpyruvic acid approach

In our second attempt to synthesize the intermediate **(20)** we adopted Steglich's approach for pyrrole synthesis as documented in the literature[37]. We were thus required to synthesize the corresponding arylpyruvic acid **(23)**, which in turn was constructed from *p*-anisaldehyde **(21)** according to the literature procedures [56]. The condensation of acetyl-glycine **(15)** with *p*-anisaldehyde **(21)** in acetic anhydride, by using sodium acetate as a base, gave intermediate **(22)**, which was subjected to acid hydrolysis to obtain the derived (4-methoxyphenyl) pyruvic acid **(23)** with a 65% overall yield.

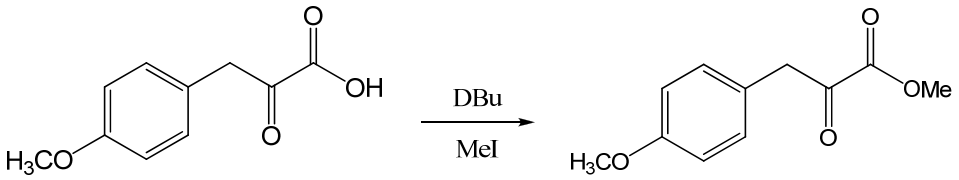



**21** **15** **22**




**23**

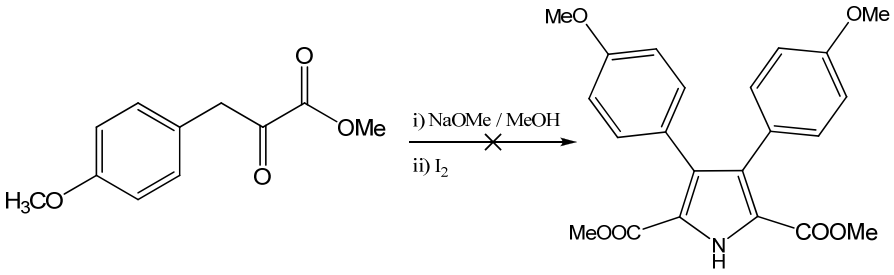
The conversion of arylpyruvic acid to the corresponding methyl ester **(24)** was achieved in DMF at a low temperature by using DBU as a base.




**24**

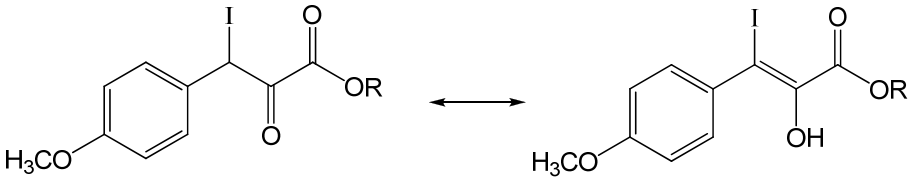
The α-keto ester "3-(4-hydroxyphenyl) pyruvat" **(24)** was proved to be very unstable and to decomposes readily at room temperature.

Base-mediated coupling of two 3-phenylpyruvate fragments, by treating them with $I_2$, NaOMe in methanol and then adding concentrated aqueous $NH_3$, gave no desired product, even by heating for 4 hours.




Different attempts, including the use of sodium bis(trimethyl silyl) amide as a base, were unsuccessful.

The failure of the aforementioned approach was attributed to the formation of the enol-form **(25)** of the ketone **(24).**



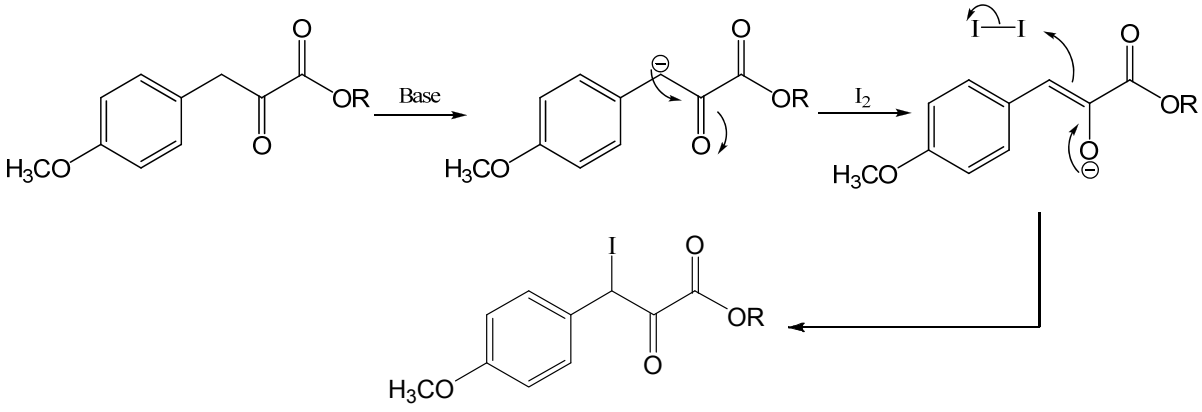

R = H, Me

**24**

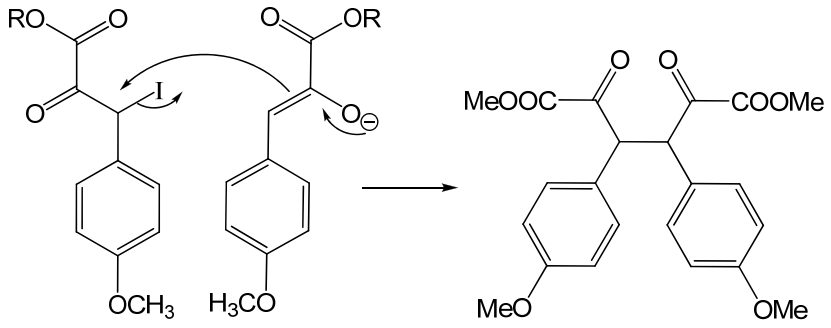
R= H, Me

**25**

The mechanism of condensation of **(23)** is believed to be initiated by the attack of enolate on $I_2$ to generate the intermediate **(26)**, which undergoes self-condensation with another enolate of **(24).**




**26**




**27**

Finally, addition of ammonia led to the cyclization and formation of the pyrrole ring.

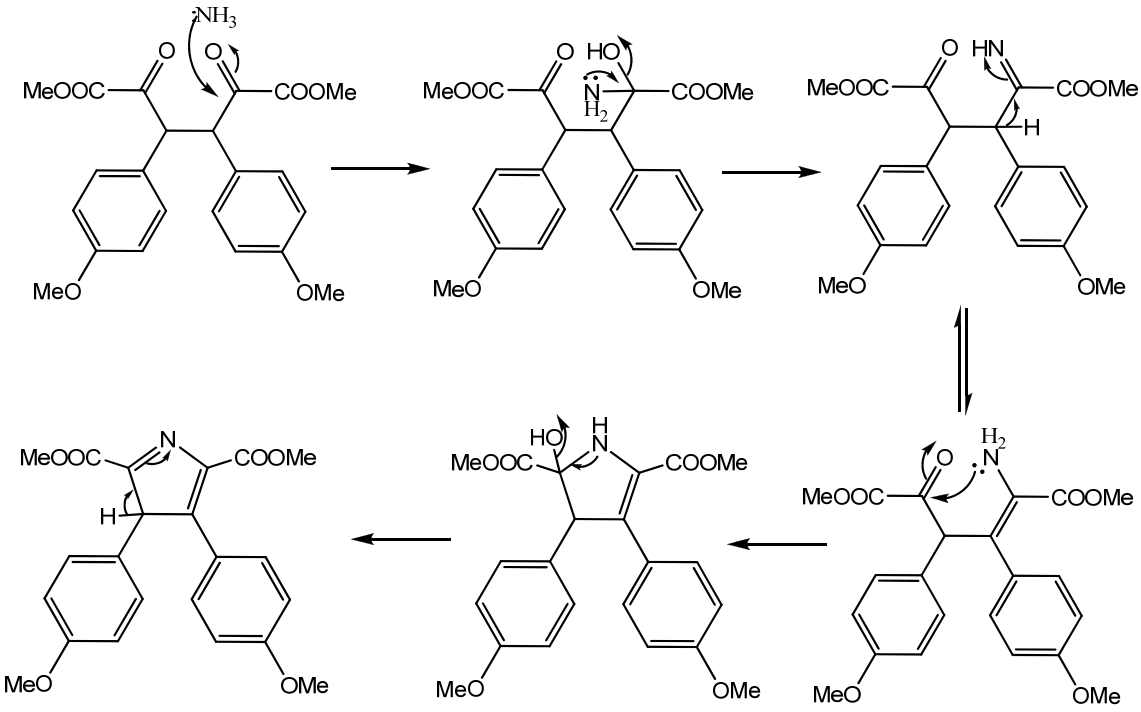
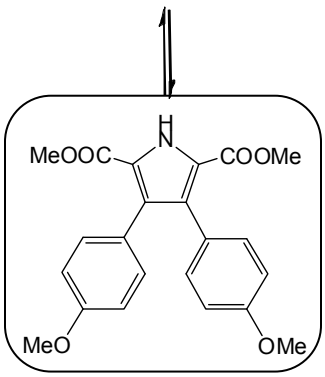


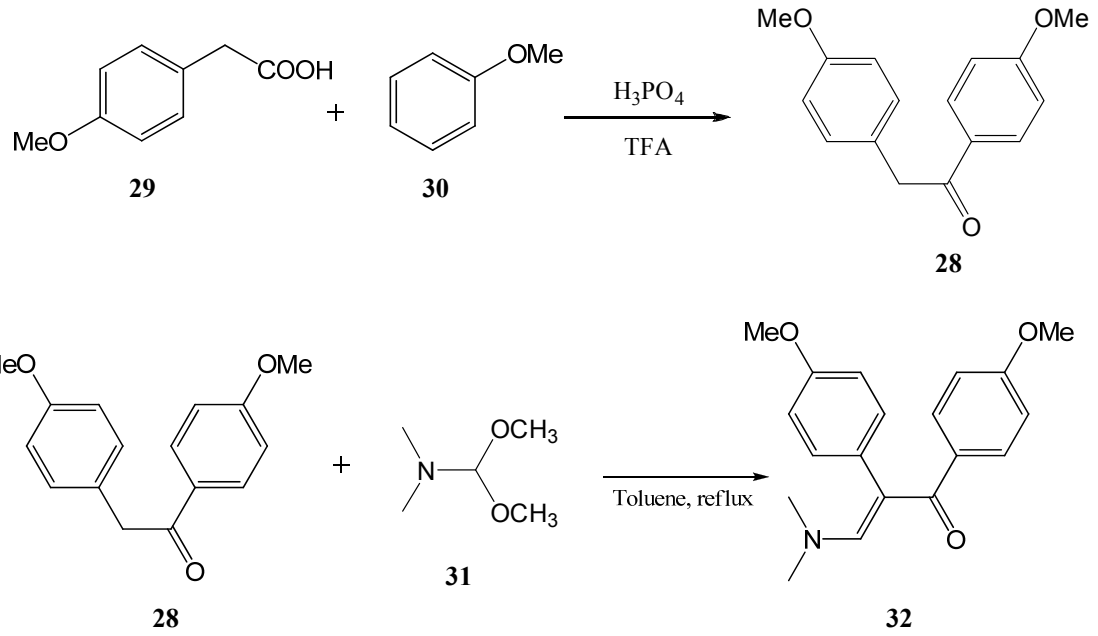

**Figure 22**



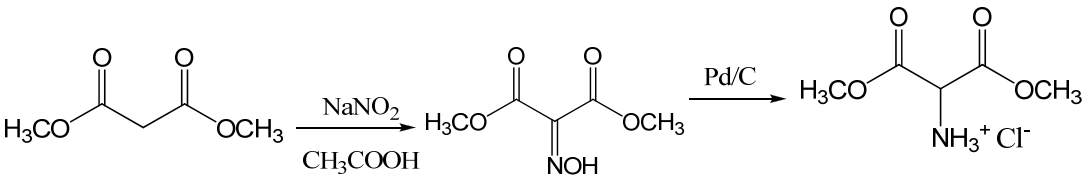
### 4.1.1.3 Vinylogous amide approach for pyrrole synthesis

As an alternative approach, we adopted vinylogous iminium chemistry to synthesize the desired pyrrole **(20)**.

The synthesis pathway was commenced by the preparation of vinylogous amide **(32)**, which in turn was synthesized from anisol **(30)** in two steps. First, the anisol **(30)** was acylated with 4-methoxyphenyl acetic acid **(29)** [57] in the presence of trifluoroacetic anhydride and phosphoric acid to generate desoxyanisoin **(28)**, giving an excellent yield (98%). Second, the desoxyanisoin was condensed with *N,N*-dimethylformamide dimethyl acetal **(31)** in refluxing toluene.

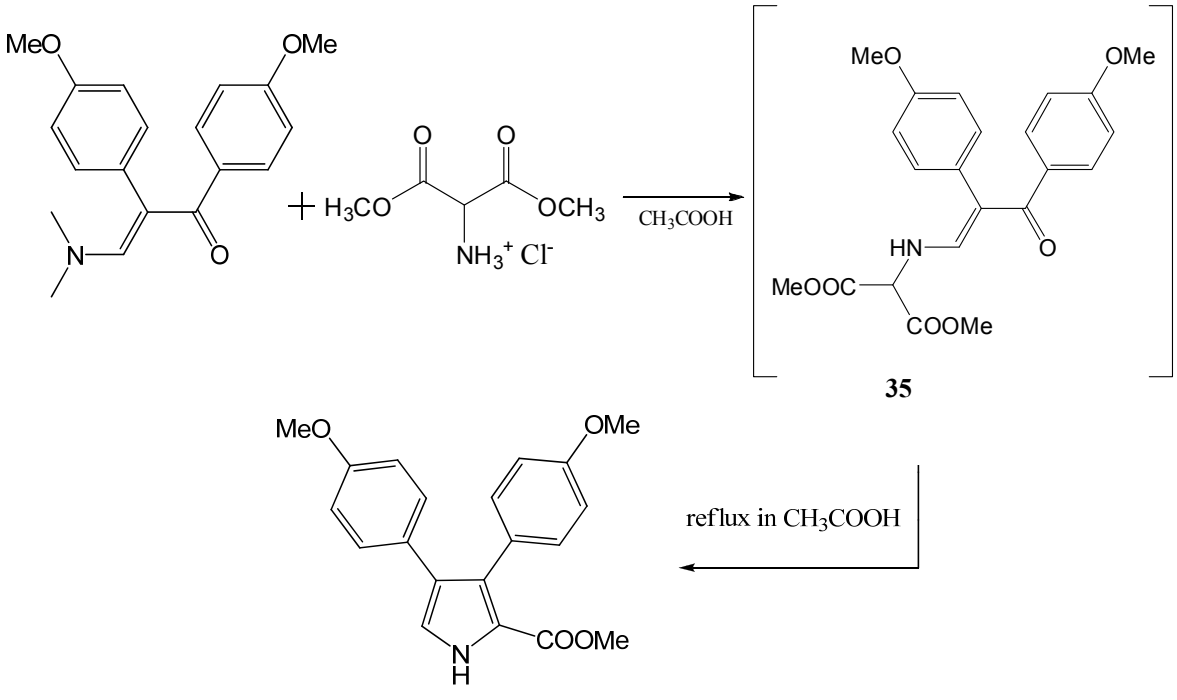



In order to get the desired key pyrrole intermediate **(20)**, we required to synthesize dimethyl aminomalonate hydrochloride **(34)** which was to be coupled with the intermediate **(32)**. Thus, the dimethyl malonate **(33)** was treated with sodium nitrite in acetic acid, followed by hydrogenation in methanolic solution of HCl using Pd/C as a catalyst to get the hydrochloride salt **(34)**.




**33** **34**

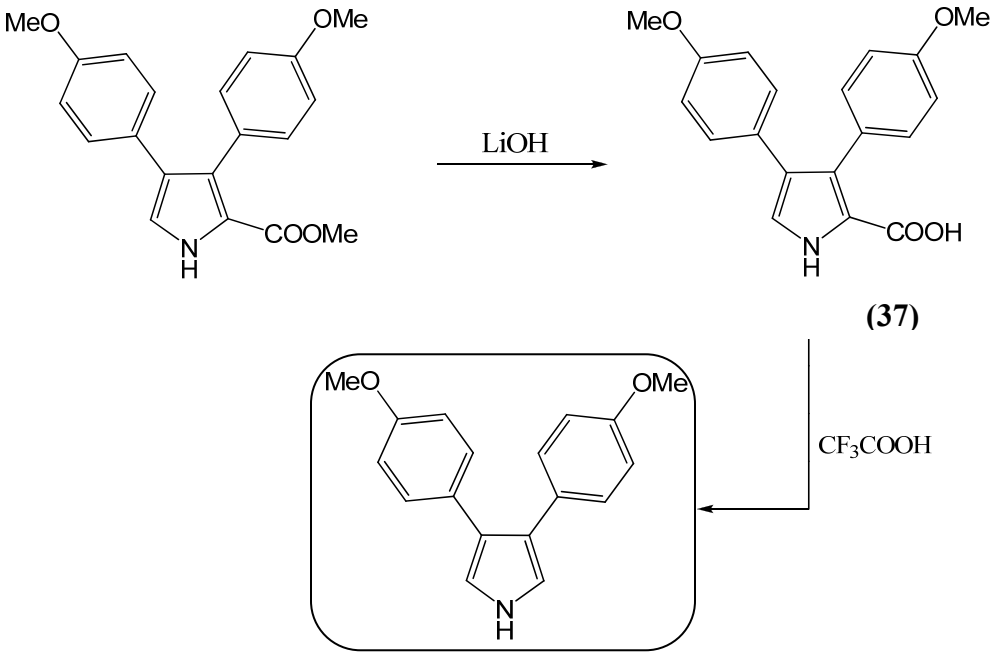
The condensation of the two key intermediates, vinylogous amide **(32)** and dimethyl aminomalonate hydrochloride **(34)** finally led us to the formation of the desired pyrrole core. The condensation of vinylogous amide **(32)** with dimethyl amino malonate **(34)** was carried out in acetic acid at a high concentration, followed by intramolecular ring closure of the proposed intermediate **(35)** and the loss of the methoxycarbonyl moiety by refluxing in acetic acid overnight [58].




**35**

**36**

The proposed way provided an easy access to the intermediate **(36)** and it gave an excellent yield. In order to transform it to the desired pyrrole intermediate **(20)**, we required two further steps: ester hydrolysis and subsequent decarboxylation.




For this purpose, ester **(36)** was hydrolyzed by LiOH to afford the corresponding acid **(37)** with a very good yield (98%). The decarboxylation of the acid derivative **(37)** was realized by using trifluoroacetic acid in $CH_2Cl_2$ at room temperature, which allowed us to access the key intermediate **(20)** in a high yield (98%)

The advantages of the revised approach can be summarized as follows.

1 - It provides an efficient way to prepare the pyrrole core in two steps in high overall yield (60%).

2 – Since the synthesis goes through unsymmetrical intermediate, tracing the formation of pyrrole **(20)** can be easily elucidated by NMR.

The reaction mechanism for the formation of pyrrole ester intermediate **(36)** is believed to be the following (Figure 23).

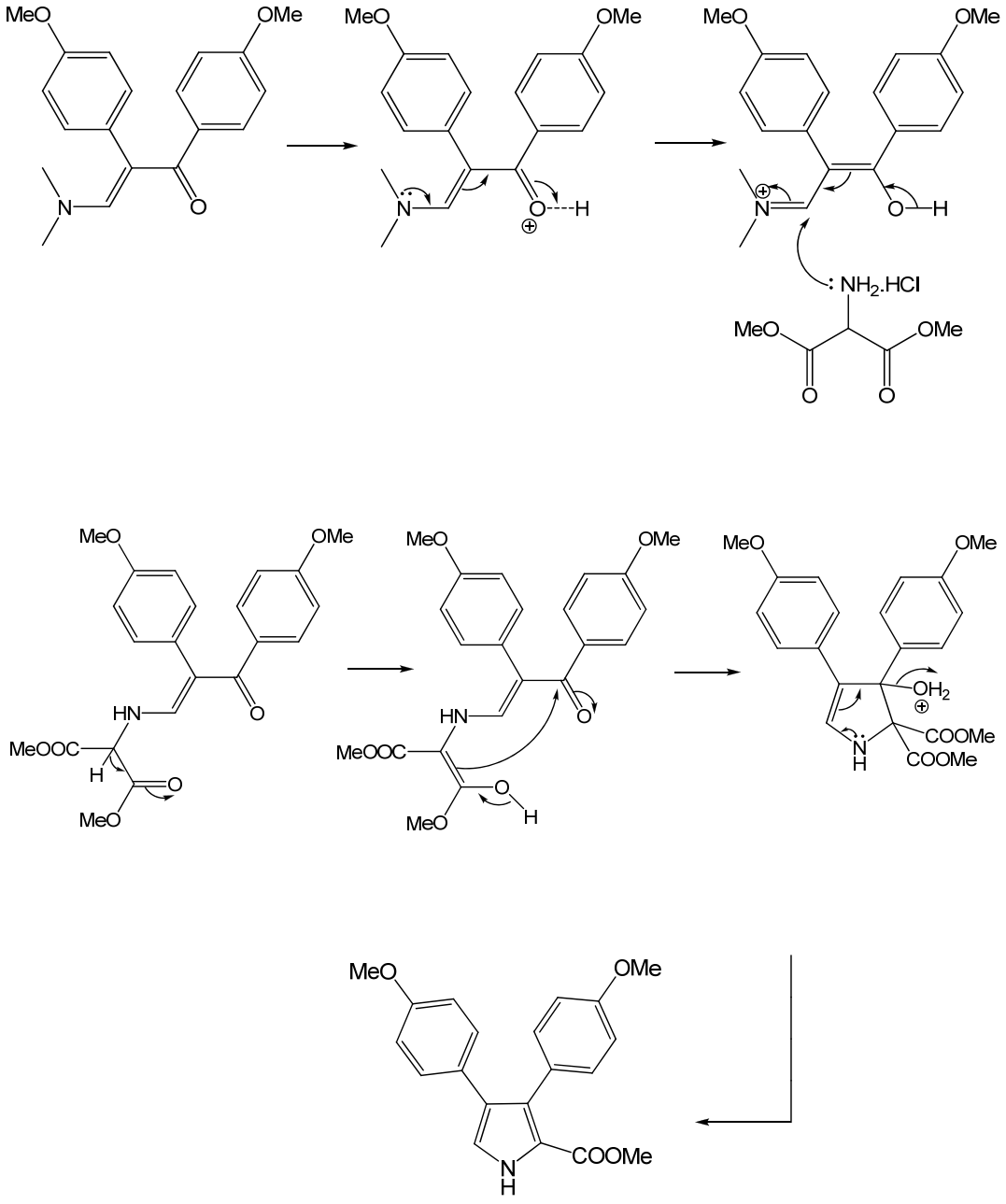


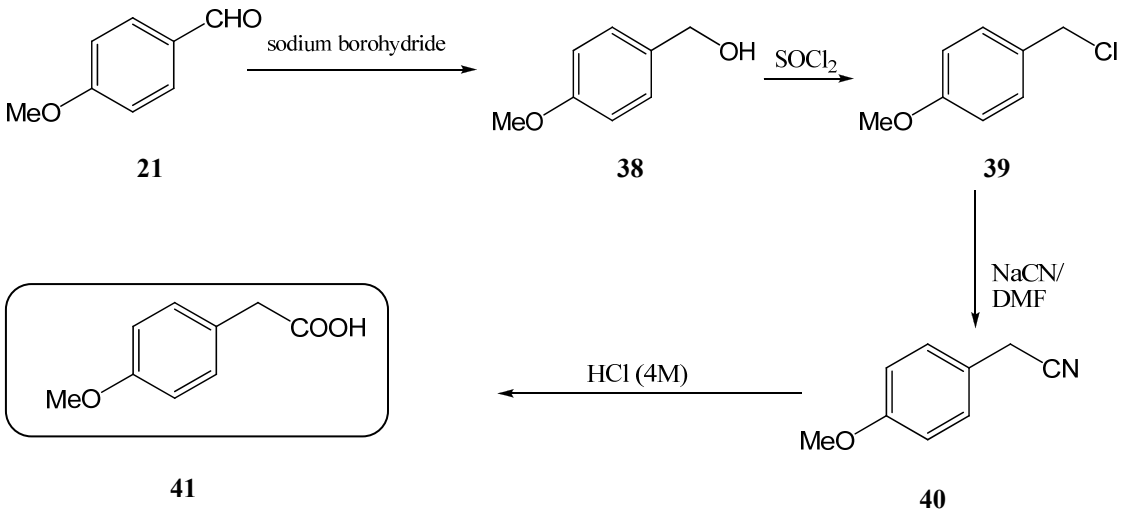

**Figure 23**

## 4.1.2 Synthesis of Neolamellarin A

After preparing the key intermediate **(20)**, we next focused on the synthesis of the corresponding acids which was to be coupled with intermediate **(20)** in order to accomplish the synthesis of neolamellarin A.

### 4.1.2.1 Synthesis of 4-methoxyphenylacetic acid

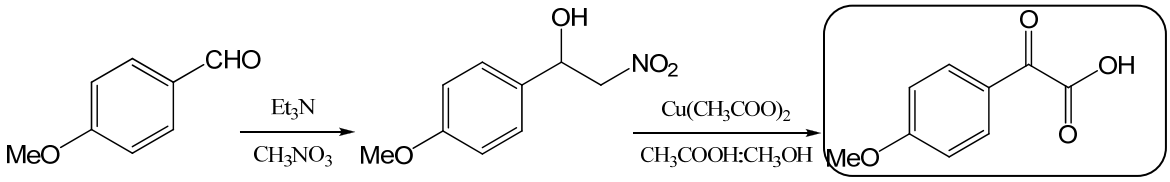
4-Methoxyphenylacetic acid **(41)** was prepared in three steps by the reduction of *p*-anisaldehyde **(21)** with sodium borohydride followed by conversion of the alcohol **(38)** to the corresponding chloride **(39)** by thionyl chloride. The reaction of NaCN with chloride **(39)** in DMF produced cyanide **(40)**, which was subjected to acid hydrolysis to obtain the desired acid **(41)**.




**21** **38** **39** **40** **41**

### 4.1.2.2 Synthesis of 4-methoxyphenyl oxoacetic acid

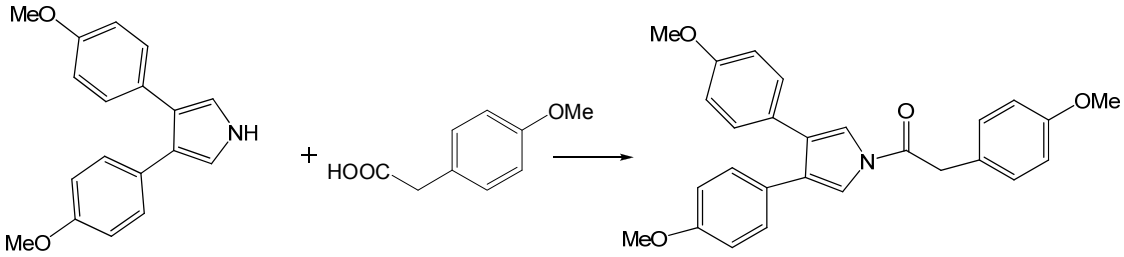
The α-keto acid **(43)** was synthesized by the Henry reaction of nitromethane with *p*-anisaldehyde **(21)** to obtain an adduct **(42)**, which was oxidized with copper acetate refluxing in a mixture $CH_3COOH$:$CH_3OH$ (1:1) to obtain the desired α-keto acid **(43)**.




**21** **42** **43**

### 4.1.2.3 Synthesis of the final target compound "Neolamellarin A"

Once the key intermediates **(20)** and **(41)** were in hand, we next advanced to the challenging step of acylating the pyrrole **(20)**.



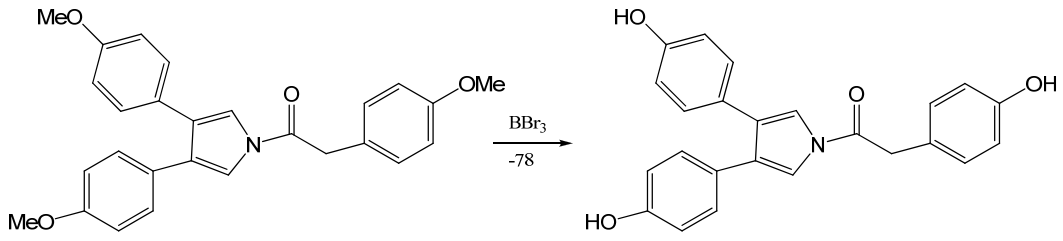

**20** **41** **44**

For this purpose, many attempts were made by acylating the acid chloride derivative of **(41),** which was prepared by using either thionyl chloride or oxalyl chloride in $CH_2Cl_2$, and using different bases such as triethyl amine, 2,6-lutidine, and sodium hydride.

The preparation of the advanced intermediate **(44)** was finally achieved when acylation of the acid chloride of corresponding acid **(41)** with pyrrole **(20)** was carried out in DMF and molecular sieves, by using 2.2 eq of sodium hydride.

The use of high temperature in the reaction was avoided, since it would have led to the decomposition of the labile acid chloride.

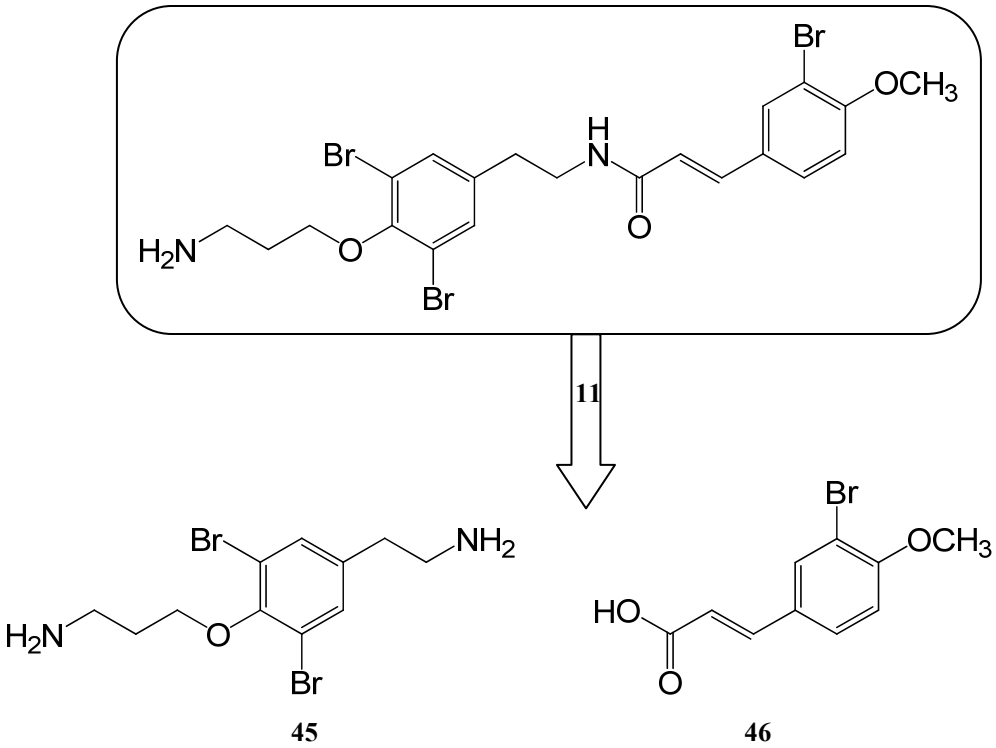
The last step in our approach was the demethylation of the phenol groups, which was achieved by using $BBr_3$ in $CH_2Cl_2$.




**44** **6**

## 4.2 Synthesis of Aplysamine 6

In our approach towards the first total synthesis of Aplysamine 6, we explored many routes to prepare the key intermediates, amine **(45)** and acid **(46),** in order to build the best synthesis pathway for our compound.



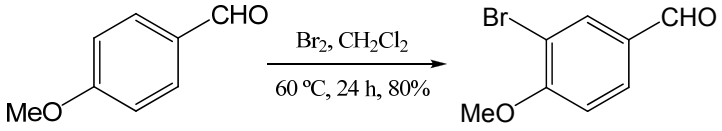

**45** **46**

In our search for the best synthetic pathway, we kept in mind the sensitivity and variety of functional groups present in the compound, which required further precautions to be applied in the reaction sequence to get the highly functionalized intermediates.

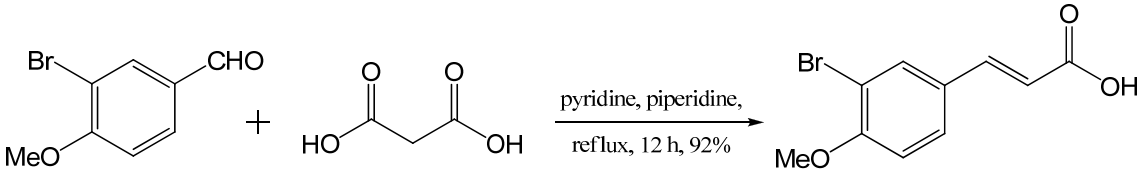
Moreover, in our synthesis approach, we aimed to utilize very common starting materials available in-house that will facilitate the large-scale synthesis of this natural product as an anti-cancer agent.

### 4.2.1 Synthesis of (3-bromo-4-methoxyphenyl) acrylic acid

Our synthetic strategy commenced with the synthesis of (3-bromo-4-methoxyphenyl) acrylic acid **(46)**. This was accomplished by the conversion of *p*-anisaldehyde **(21)** to the known bromobenzaldehyde **(47)** that required an extended reaction time of 24 hours, stirring with $Br_2$ in dichloromethane at 60°C. In turn, bromo derivative **(47)** was subjected to Doebner–Knoevenagel condensation with malonic acid in the presence of pyridine and piperidine to afford cinnamic acid **(46)** with a 92% yield.



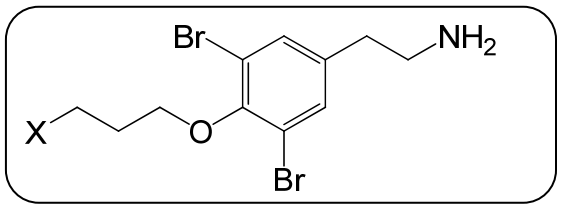

**21** **47**



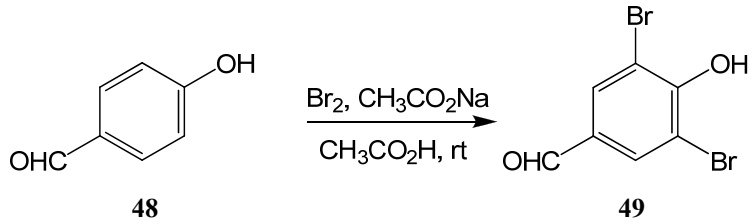

**47** **46**

### 4.2.2 Synthesis of the intermediate (45)

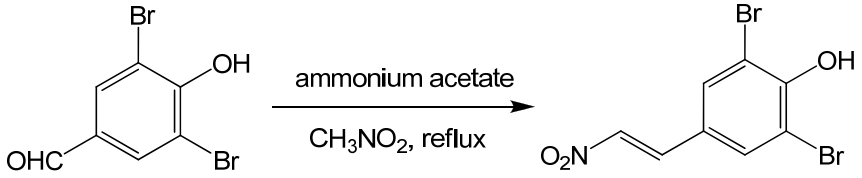
Starting from 4-hydroxybenzaldehyde **(48)**, three modifications were necessary to access our target intermediate. These were: the formation of 2-aminoethyl group, the bomination of the aromatic compound, and the alkylation of the phenol group.



The synthesis of the intermediate **(45)** was started with bromination of the aromatic aldehyde **(48)** by stirring with $Br_2$ in acetic acid at room temperature to get the 2,6-dibromo aldehyde **(49)**. This in turn was refluxed with nitromethane by using ammonium acetate as the base to construct the intermediate **(49)**.

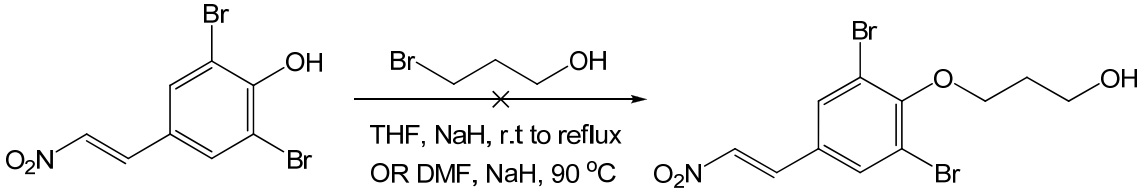



**48** **49**



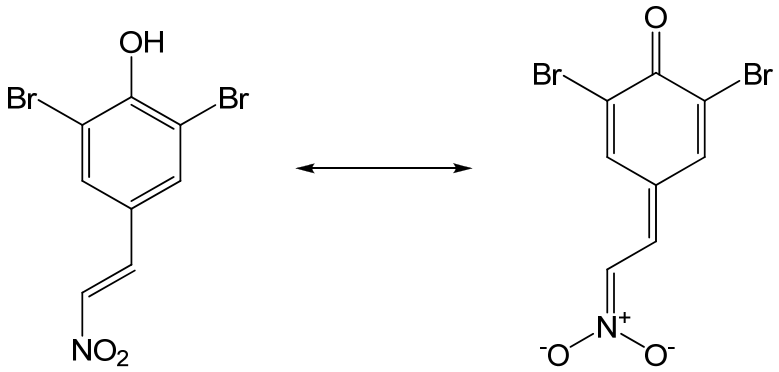

**49** **50**

The alkylation of intermediate **(50)** to synthesis **(51)** proved to be unsuccessful. The reaction was carried out at elevated temperature by using different solvents such as THF or DMF, and NaH as a base.



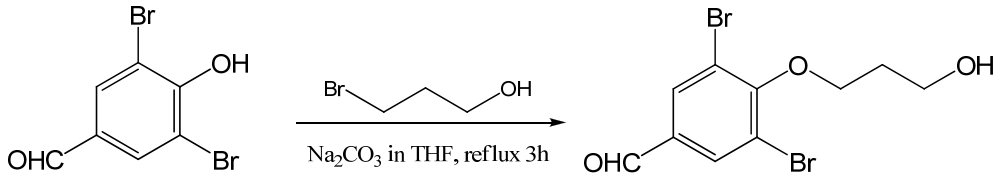

**50** **51**

The low reactivity of the phenol **(50)** was attributed to tautomeric quinone form **(52)** that depresses the reactivity of the phenol group towards the alkylation reaction.

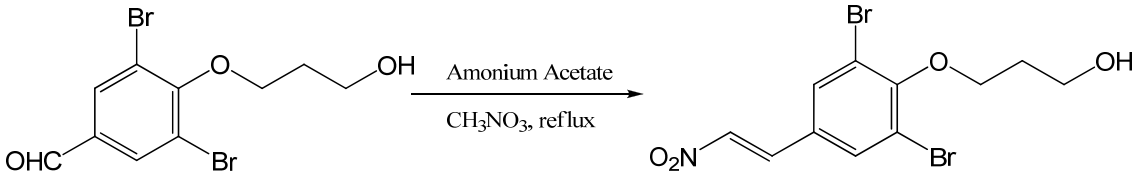



**50** **52**

Thus, we decided to alkylate the phenol group prior to the Henry reaction on the substrate **(49).** Alkylation of aldehyde **(49)** with 3-bromopropanol was carried out in THF by using potassium carbonate as a base to get the alkylated product **(53)**. This was then subjected to the Henry reaction under the same conditions as previously described.

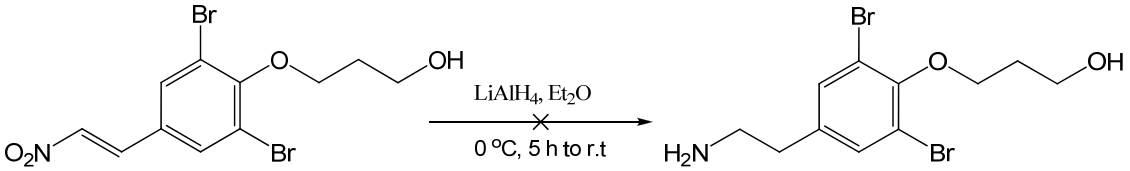



**49** **53**



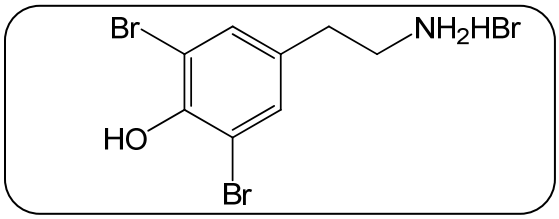

**53** **54**

The reduction of alcohol **(54)** with $LiAlH_4$ proved to be problematic, since it yielded a mixture of reduced mono and di-debrominated products.




**54** **55**

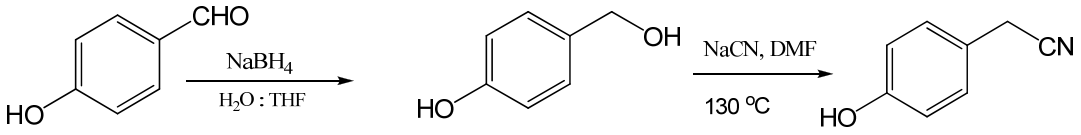
Consequently we changed our strategy by first synthesizing the amino moiety and then brominating the aromatic ring to get access to the intermediate **(56)**.




**56**

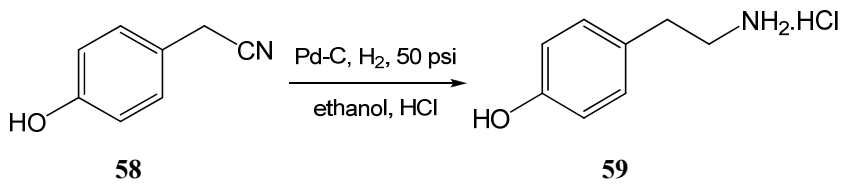
### 4.2.3 Synthesis of 4-(2-aminoethyl)-2,6-dibromophenol hydrobromide

The synthesis of the phenol **(56)** began with reduction of aldehyde **(48)** with $NaBH_4$ to get the corresponding alcohol **(57),** which was heated with NaCN in DMF at 130°C to get the intermediate **(58)**.

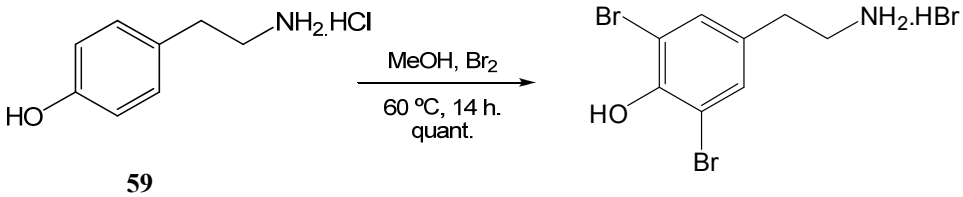



**48** **57** **58**

Hydrogenation of **(58)** in a mixture of EtOH and aqueous HCl produced the aminohydrochloride **(59)** with a 95% yield.




**58** **59**

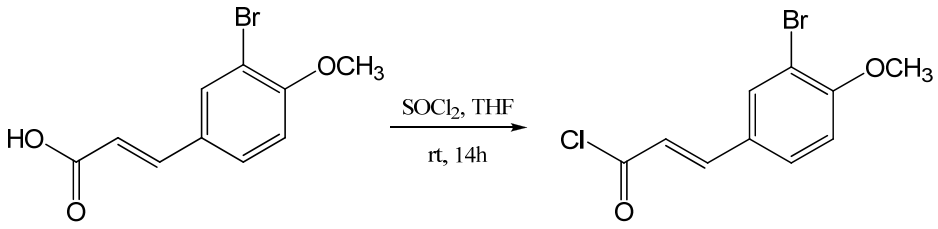



**59** **60**

The bromination of 4-hydroxyphenethylamine **(59)** was carried out in methanol at 60°C to produce the corresponding dibromide **(60)** as an off-white solid in a quantitative yield.

## 4.2.4 Final steps of synthesis of Aplysamine 6

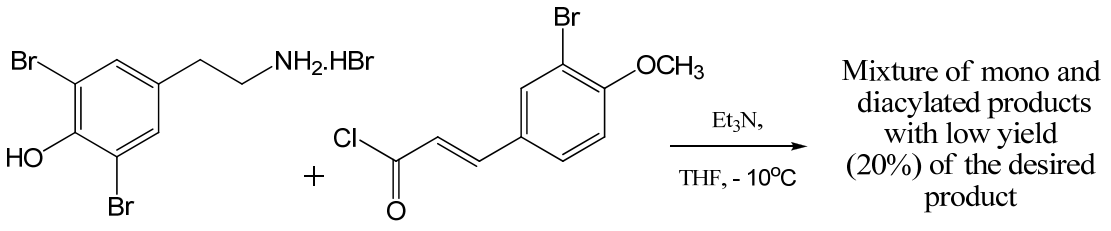
Having the desired dibromide in hand, we next focused on the chemoselective acylation of dibromide **(60)**. The acid **(46)** was transformed into the corresponding acid chloride **(61)** with the aid of thionyl chloride in THF.




**46** **61**

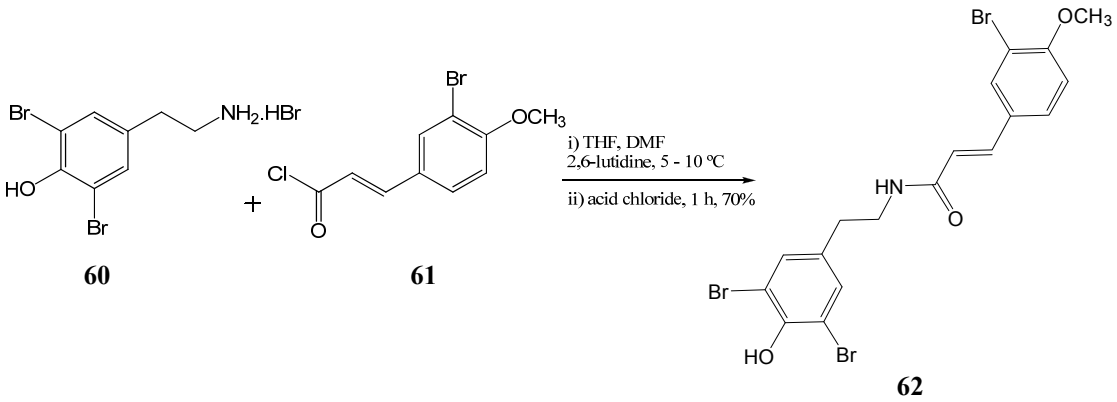
After evaporation of the solvents, a solution of acid chloride **(61)** in dry THF was added dropwise to a solution of dibromide **(60)** in THF at -10°C using triethylamine as base. The reaction was unsuccessful due to the low yield (20%) of the desired amide

**(62)**, along with the formation of a diacylated side-product which made column purification very tedious.



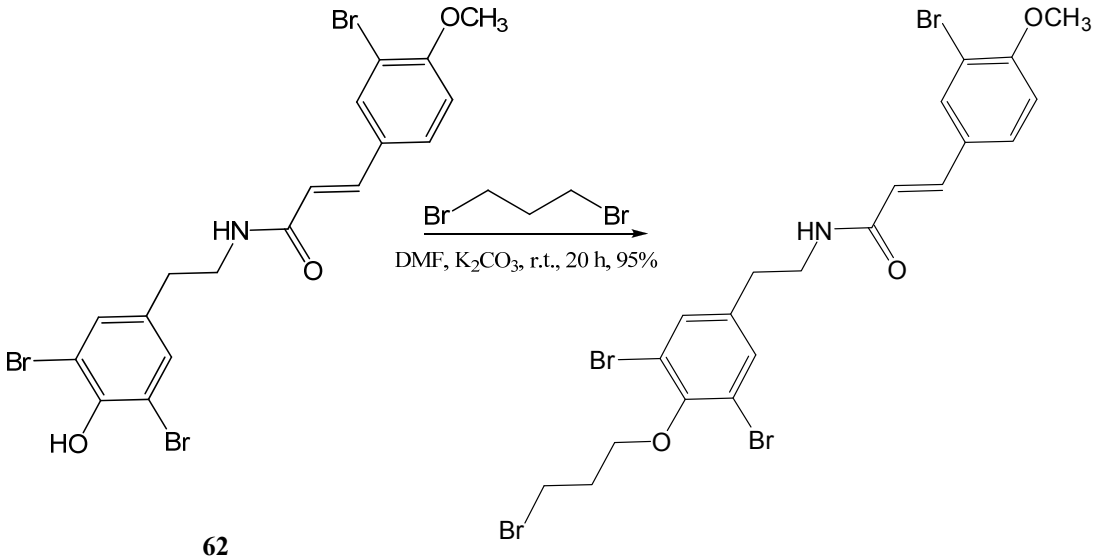

**60** **61**

The solubility of the dibromide **(60)** in THF at -10°C was evidently very poor. Hence, in a second attempt, we added the acid chloride **(61)** to a solution of dibromide **(60)** in a mixture of THF and DMF (1:1) at 5 to 10°C using 2,6-lutidine as the base. This gave the desired amide **(62)** cleanly with a 70% yield after column purification.



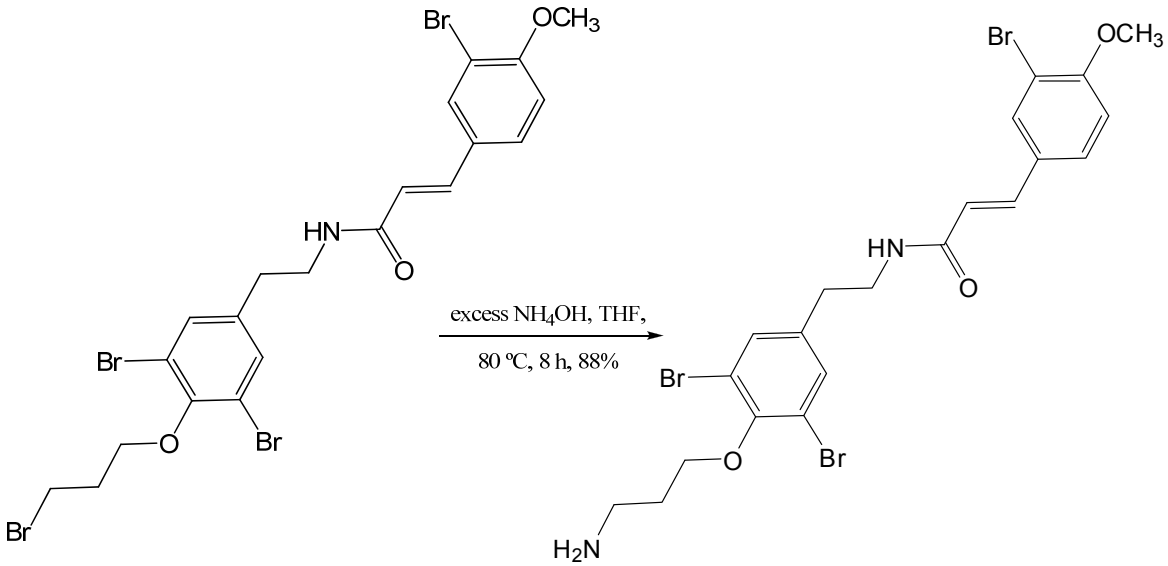

**60** **61** **62**

The amide **(62)** was alkylated by the action of excess 1,3-dibromopropane in DMF by using potassium carbonate as the base to give the compound **(63)** with an excellent yield (95%).




**62** **63**

In turn, the compound **(63)** was reacted with excess ammonium hydroxide at 80°C for 8 h in a sealed tube to produce aplysamine 6 **(11)** with an excellent yield (88%). The spectral data of our synthetic **(11)** coincided with those of the natural material [54].




**63** **11**

In conclusion, we accomplished an efficient first total synthesis of Aplysamine 6, an inhibitor of isoprenylcysteine carboxy methyltransferase, with an overall 55% yield, starting from **(46)** and **(60)**.

Figure 24 outlines the complete synthesis scheme of Aplysamine 6 [59].

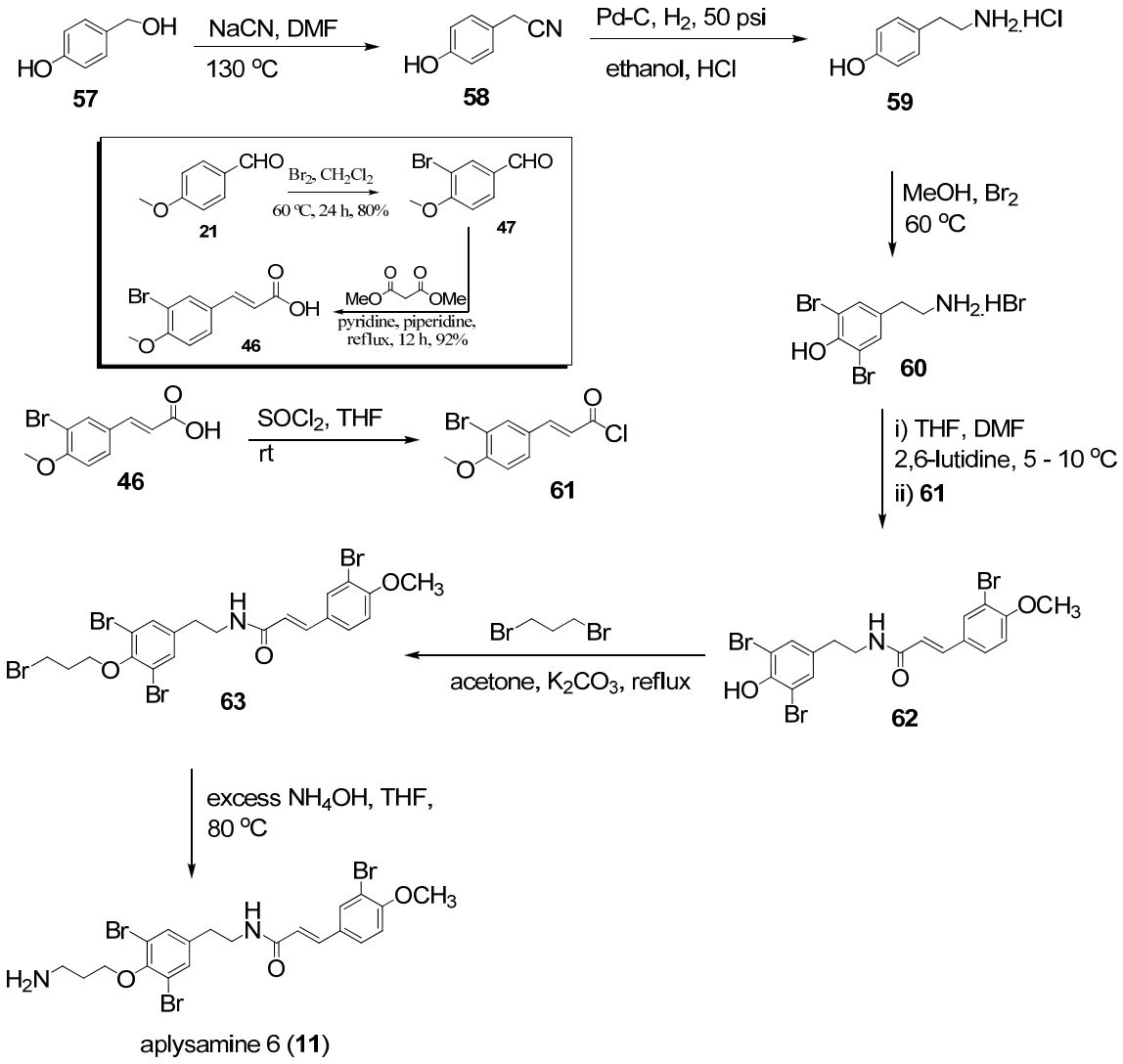



**Figure 24**

# CHAPTER 5

# EXPERIMENTAL WORK

## 5.1 Instrumentation and Chemicals

$^{1}H$ NMR and $^{13}C$ NMR spectra were recorded on a JEOL Lambda 500 MHz spectrometer. Chemical shifts were reported in ppm (δ) relative to tetramethyl silane (TMS) by using ($CDCl_3$) or (DMSO) as deuterated solvents. Multiplicities were reported as singlet (s), doublet (d), triplet (t), quartet (q) or multiplet (m), and coupling constants (J) were reported in hertz (Hz). IR Spectra were recorded on a Nicolet™ 6700 FT-IR spectrometer from a thermo-electron by using a smart orbit for net samples, and they were reported in wave numbers ($cm^{-1}$) (Spectral resolution, 4 $cm^{-1}$; Number of scans, 4). Mass was determined by using Agilent 7000A Triple Quadrupole GC/MS. Elemental analysis was carried out on a EuroVector Elemental Analyzer Model EA3000. Al mps are uncorrected.

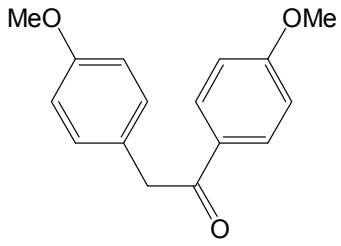
Thin layer chromatography (TLC) was frequently used to monitor reactions and to give qualitative determination of sample purity. TLC analyses were performed on silica gel Merck 60 F254 plates, and spots were visualized under a spectroline UV lamp operating at short and long wavelength ranges. Visualization was improved by dipping plates into a phosphomolybdic acid solution, and then by drying in a blast of hot air. Purification of the products was carried out either by recrystallization or by flash column chromatography. The column was packed with Silica gel 100 from Fluka Chemie AG (Buchs, Switzerland). Ethyl acetate, petroleum ether (boiling fraction 60-80) and hexane were used as eluting solvents, in volume-by-volume ratios as stated.

Chemicals were purchased from commercial sources, and they were used without any further purification unless otherwise specified. All solvents were of reagent grade, and dichloromethane was passed through alumina before use. Specially dried

(anhydrous) solvents were used where necessary. Glassware for moisture-sensitive reactions were oven dried at 120-140 °C for at least three hours and cooled in a desiccator prior to use. Some of the reactions were run in an inert atmosphere of nitrogen as stated.

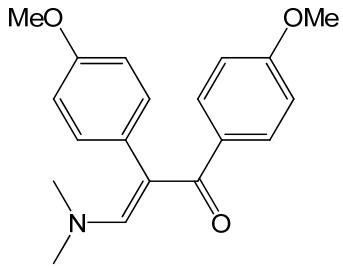
## 5.2 Synthesis of Neolamellarin A

#### 5.2.1.1 1,2-bis(4-methoxyphenyl)ethanone (Desoxyanisoin) (28)



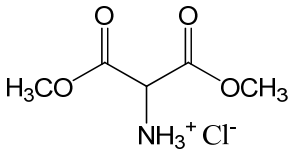

To a mixture of 4-methoxyphenyl acetic acid **(29)** (0.6 g, 3.6 mmol), anisol **(30)** (0.46 g, 4.3 mmol), and 88–93% phosphoric acid (3.6 mmol) was rapidly added trifluoroacetic anhydride (12.1 mmol) with vigorous stirring at 25 °C. The mixture turned into a dark-colored solution with a vigorous exothermic reaction. The reaction mixture was stirred for 1 min at the same temperature, and ice-cold water (50 mL) was added. The off-white solid formed was filtered and washed with cold hexane (2 × 10 mL) to obtain **(28)** as an off-white solid (0.89 g, 97%); mp: 105-106 °C; IR (Neat) $\nu_{max}$ 3000, 2952, 2902, 2832, 1673, 1597, 1505, 1455, 1410, 1327, 1240, 1166, 1106, 1023, 983, 816 $cm^{-1}$; $^{1}H$ NMR ($CDCl_3$): δ 3.78 (s, 3H), 3.86 (s, 3H), 4.18 (s, 2H), 6.84 (d, 2H, *J* = 8.85), 6.91 (d, 2H, *J* = 8.85), 7.16 (d, 2H, *J* = 8.55), 7.98 (d, 2H, *J* = 8.85). $^{13}C$ NMR ($CDCl_3$): δ 44.38, 55.24, 55.47, 113.76, 114.10, 126.93, 129.61, 130.37, 130.93, 132.32, 158.44, 163.47, 196.60. MS (*m/z*): 256 ($M^+$), 135, 121, 107, 92, 77, 63, 39.

### 5.2.1.2 3-Dimethylamino-1,2-bis-(4-methoxy-phenyl)-propenone (Vinylogous amide) (32)




To a solution of α-aryl ketone **(28)** (0.67 g, 2.64 mmol) in toluene (14 mL) was added N,N-dimetylformamide dimethyl acetal **(31)** (1.4 mL, 10.56 mmol). The solution was heated overnight, and the solvent was removed under reduced pressure to yield a yellow solid, which was recrystallized by hexane/ethyl acetate (4:1) to afford vinylogous amide **(32)** (0.80 g, 98%) as a white solid; mp: 115-116 °C; IR (Neat) $\nu_{max}$ 2930, 2833, 1621, 1581, 1552, 1234, 1168, 1022, $cm^{-1}$; $^{1}H$ NMR ($CDCl_3$): δ 2.74 (s, 6H), 3.79 (s, 6H), 6.76 (d, 2H, *J* = 8.8), 6.80 (d, 2H, *J* = 8.85), 7.05 (d, 2H, *J* = 8.85), 7.36 (s, 1H), 7.42 (d, 2H, *J* = 8.8). $^{13}C$ NMR ($CDCl_3$): δ 43.44, 55.18, 55.23, 111.29, 112.77, 113.16, 129.96, 131, 133, 134.18, 153.09, 157.98, 160.57, 194.02. MS (*m/z*): 311 ($M^+$), 294, 135, 77.

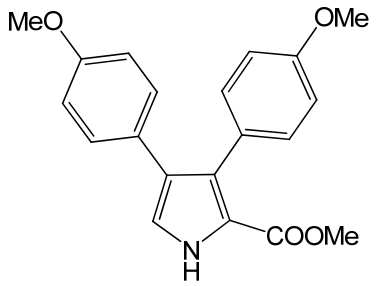
### 5.2.1.3 Dimethyl aminomalonate hydrochloride (34)




To a mixture of dimethyl malonate **(33)** (4 g, 30.27 mmol) and acetic acid (5.18 ml, 90.82 mmol) in water (7 ml) at 0 °C was added sodium nitrite (6.27 g, 90.82 mmol). The reaction mixture was stirred at room temperature for 3 h, water was added, and the mixture was extracted four times with diethyl ether. The combined ether layers were

cautiously treated with an equal volume of saturated aqueous sodium bicarbonate. Solid sodium bicarbonate was then added until the aqueous layer turned to yellow. The ether layer was separated and dried over sodium sulfate, and it was concentrated under reduced pressure to give crude oxime. Without any further purification, the crude oxime was dissolved in EtOH (31 ml) and transferred to a Parr pressure vessel which was charged with 10% palladium on charcoal (1.18 g) and methanolic-HCl (3N, 14 ml). The mixture was subjected to hydrogenation at (50 psi) for 12 h. The reaction mixture was filtered through a pad of Celite® and rinsed thoroughly with ethanol. Removal of the solvent under reduced pressure and reconcentration by $CH_2Cl_2$ gave pure **(34)** (5.06g, 91%) as a green solid. mp: 132-134 °C; IR (Neat) $\nu_{max}$ 3330 (br), 2959, 1739, 1435, 1268 $cm^{-1}$; $^1H$ NMR (DMSO): δ 3.77 (s, 6H), 5.06 (s, 1H), 9.25 (s, 3H). $^{13}C$ NMR (DMSO): δ 53.76, 54.54, 164.17. MS (*m/z*): 147 ($M^+$), 88, 60, 33.

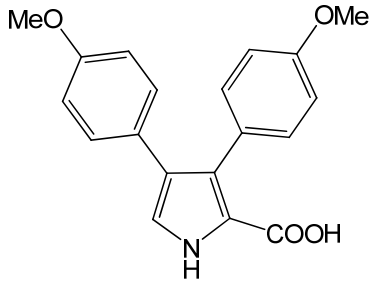
#### 5.2.1.4 3,4-Bis-(4-methoxyphenyl)-1*H*-pyrrole-2-carboxylic acid methyl ester (36)




To a solution of of vinylogous amide **(32)** (0.76 g, 2.44 mmol) in glacial acetic acid (4.5 ml) was added dimethyl aminomalonate hydrochloride **(34)** (0.456 g, 2.49 mmol). The resulting mixture was stirred at room temperature under $N_2$ atmosphere for 1 h. The mixture was then diluted with (120 mL) of glacial acetic acid, and it was heated to reflux for 12 h. After the mixture was cooled to ambient temperature, the acetic acid was removed under reduced pressure. The residue was taken up in $CH_2Cl_2$ (50 mL) and washed with saturated sodium bicarbonate solution. The organic layer was washed with brine and dried over anhydrous $Na_2SO_4$. Final purification by column chromatography

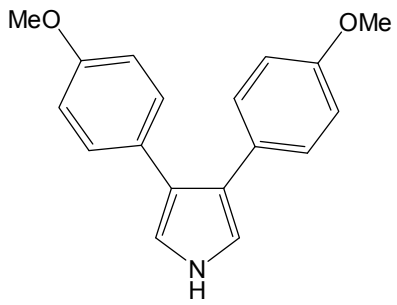
using hexane/ethyl acetate (4 : 1 to 3 : 1) as eluent afforded pure **(36)** (0.51 g, 62%) as yellow needles. mp: 172 - 175 °C; IR (Neat) $\nu_{max}$ 3301, 1669, 1439, 1378, 1242, 1169 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 3.73 (s, 3H), 3.76 (s, 3H), 3.82 (s, 3H), 6.75 (d, 2H, *J* = 8.85), 6.85 (d, 2H, *J* = 8.85), 7.02 (s, 1H, obscured by the next peak), 7.03 (d, 2H, *J* = 8.55), 7.19 (d, 2H, *J* = 8.55), 9.17 (br s, 1H). $^{13}C$ NMR ($CDCl_3$): δ 51.29, 55.14, 55.17, 113.08 (2C), 113.64 (2C), 119.33, 120.07, 126.38, 126.45, 127.04, 129.04, 129.44 (2C), 131.85 (2C), 157.99, 158.49, 161.57. MS (*m/z*): 337 ($M^+$), 305, 262, 191, 152.

### 5.2.1.5 3,4-Bis(4-methoxyphenyl)-1*H*-pyrrole-2-carboxylic acid (37)



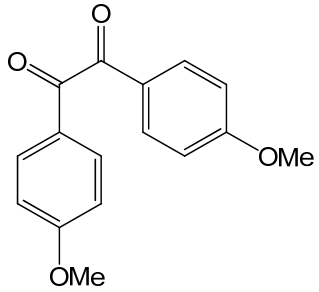

To a solution of the methyl ester **(36)** (0.62 g, 1.84 mmol) in a mixture of THF (8 ml), $CH_3OH$ (8 ml) and $H_2O$ (4 ml) was added LiOH (0.46 g, 11.05 mmol) and the mixture was stirred overnight at room temperature. The solvents were removed under reduced pressure, and the mixture was diluted with water and extracted with diethyl ether three times. The water layer was separated and acidified with HCl (1N) to pH 2 and extracted with $CHCl_3$ (3 × 20ml). The organic layer was dried over sodium sulfate and evaporated to give the desired product **(37)** (0.56 g, 95%) as a white solid.$^1H$ NMR (DMSO): δ 3.66 (s, 3H), 3.73 (s, 3H), 6.72 (d, 2H, *J* = 8.55), 6.81 (d, 2H, *J* = 8.55), 6.94 (d, 2H, J = 8.25), 7.05 (d, 2H, *J* = 8.25), 7.08 (d, 1H, *J* = 2.75), 11.74 (s, 1H). $^{13}C$ NMR ($CDCl_3$): δ 54.89 (2C), 112.89 (2C), 113.57 (2C), 119.69, 120.56, 124.67, 127.29, 127.43, 127.52, 128.84 (2C), 131.70 (2C), 157.29, 157.78, 162.01.

### 5.2.1.6 3,4-Bis(4-methoxyphenyl)-1*H*-pyrrole (20)




To a solution of the acid **(37)** (0.50 g, 1.55 mmol) in $CH_2Cl_2$ (30 ml) was added trifluoroacetic acid (6 ml) dropwise at 0 °C. The reaction mixture was stirred overnight at room temperature, and the solvent was evaporated under reduced pressure. Ethyl actate (50 ml) was added and successively washed with (1N) NaOH (20 ml) brine (20 ml), and then dried over sodium sulfate and evaporated. The product was purified by column chromatography using hexane/ethyl acetate (4 : 1 to 3 : 1) as eluent to give **(20)** (0.42 g, 98%) as a yellow solid. mp: 104 - 106 °C; IR (Neat) $\nu_{max}$ 3446, 2843, 1493, 1236, 1017, 835, 786 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 3.79 (s, 6H), 6.81 (d, 4H, $J$ = 8.85), 6.84 (d, 2H, $J$ = 2.75), 7.19 (d, 4H, $J$ = 8.85), 8.23 (br s, 1H). $^{13}C$ NMR ($CDCl_3$): δ 55.21, 113.64, 116.75, 123.13, 128.41, 129.60, 157.84. MS (*m/z*): 279 ($M^+$), 264, 204, 165.

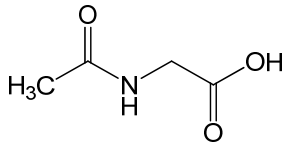
### 5.2.1.7 1,2-Bis(4-methoxyphenyl)ethane-1,2-dion (18)




To the solution of *p*-anisaldehyde **(21)** (20 g, 146.9 mmol) in a mixture of EtOH:$H_2O$ (2:1, 150 ml) was added potassium cyanide (3.82 g, 58.76 mmol) and the reaction was refluxed overnight. The solution was poured over water and extracted with

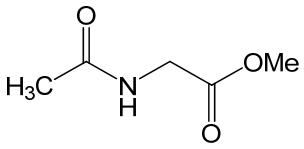
dichloromethane, yielding the coupled α-hydroxyketone **(17)**, which was dissolved in pyridine (125 ml) then $H_2O$ (75 ml) and 22.95 g $CuSO_4$ was added. After refluxing for 16 h, the reaction was cooled to room temperature and extracted with ethyl acetate. The organic layer was washed successively with HCl (1N) and brine. The solvent was reduced by evaporation, and it was left for crystallization to give **(18)** (26.20 g, 66%) as a pale yellow solid. mp: 124 – 127 °C; IR (Neat) $\nu_{max}$ 2953, 2845, 1650, 1594, 1257, 1010, 826 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 3.89 (s, 6H), 6.96 (d, 4H, *J* = 8.85), 7.95 (d, 4H, *J* = 8.85). $^{13}C$ NMR ($CDCl_3$): δ 55.65, 114.29, 126.29, 132.39, 164.86, 193.51.

### 5.2.1.8 2-acetamidoacetic acid (acetyl-glycine) (15)




To the solution of glycine **(14)** (10 g, 133.33 mmol) in water (40 ml) was add acetic anhydride (27 ml) and the reaction mixture was stirred for 1 h at room temperature. The solvent was removed under reduced pressure to give **(15)** (15.3 g, 98 %) as white crystalline solid. mp: 204 - 206 °C; IR (Neat) $\nu_{max}$ 3348, 1947, 1717, 1552, 1231, 990 $cm^{-1}$; $^1H$ NMR (DMSO): δ 1.83 (s, 3H), 3.70 (d, 2H, *J* = 5.8), 8.16 (t, 1H), 12.50 (br s, 1H). $^{13}C$ NMR (DMSO): δ 22.34, 40.66, 169.70, 171.49.

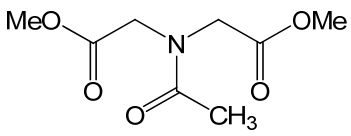
### 5.2.1.9 Methyl 2-acetamidoacetate (16)




To the solution of N-acetyl-glycine **(15)** (4 g, 34.19 mmol) in methanol (24 ml) at 0 °C was added dropwise thionyl chloride (5 ml, 68.38 mmol). The reaction was stirred

overnight at room temperature, and the solvents were removed under reduced pressure to get **(16)** (4.39 g, 98 %) as white gum. IR (Neat) $\nu_{max}$ 3349, 2341, 1719, 1383, 1231, 680 $cm^{-1}$; $^{1}H$ NMR (DMSO): δ 1.83 (s, 3H), 3.59 (s, 3H), 3.78 (d, 2H, *J* = 5.5), 8.36 (s, 1H). $^{13}C$ NMR (DMSO): δ 22.23, 40.58, 51.66, 169.85, 170.52.

### 5.2.1.10 Dimethyl *N*-acetyliminodiacetate (12)



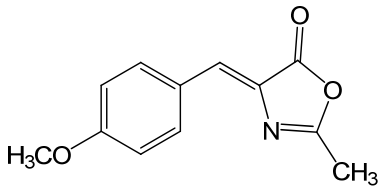

To a solution of methyl 2-acetamidoacetate **(16)** (3.2 g, 24.44 mmol) in DMF (112 ml) was added NaH (0.76 g, 31.77 mmol) at 0 °C under nitrogen atmosphere. After stirring for half an hour, methyl 2-bromoacetate (5.61 g, 36.66 mmol) was added dropwise at 0 °C, followed by the addition of NaI (0.37 g, 2.44 mmol). Stirring was continued overnight at room temperature. The reaction mixture was diluted with water and extracted with diethyl ether (50 ml). The organic layer was washed with saturated sodium bicarbonate (20 ml) and brine (20 ml), and it was dried over sodium sulfate and evaporated to get the desired product (4.35 g, 88%) as thick yellow liquid. IR (Neat) $\nu_{max}$ $cm^{-1}$; $^{1}H$ NMR ($CD_2Cl_2$): δ 1.47 (s, 3H), 3.54 (s, 4H), 3.59 (s, 6H). $^{13}C$ NMR (DMSO): δ 51.23, 54.19, 54.44, 171.02, 172.22.

### 5.2.1.11 General procedure for the preparation of 3,4-Bis(4-methoxyphenyl)-1*H*-pyrrole (20) by Hinsberg-type pyrrole synthesis

Sodium (6 g) was dissolved in 28 mL of absolute methanol under an atmosphere of nitrogen in a 250 mL three-necked flask equipped with a nitrogen inlet and reflux condenser. The solution was cooled to room temperature, and 2.26 g of dimethyl N-acetyliminodiacetate **(12)** (11.13 mmol) dissolved in 8 mL of dry methanol was added to the sodium methoxide solution while the mixture was being stirred by means of a

mechanical stirrer. Solid 3,3′-dimethoxybenzyl **(18)** (2 g, 7.42 mmol) was then added in fractions, and stirring was continued at room temperature until all of the 3,3′-dimethoxybenzyl was dissolved. The reaction mixture was slowly heated to the boiling point, and then refluxed overnight. After removal of the solvent under reduced pressure, a mixture of $H_2O$ (28 ml) and THF (28 ml) was added followed by the addition of LiOH (1.87 g, 44.52 mmol). The reaction mixture was refluxed overnight. The reaction was diluted with water and extracted with diethyl ether to remove any traces of the starting materials. The water layer was acidified with HCl (1N) to pH 2 and extracted with $CHCl_3$ (3 × 50 ml). The organic layer was dried over sodium sulfate and evaporated to get the diacid as a white needle which was used in the next step without any further purification. The diacid was refluxed under nitrogen in 25 mL of freshly distilled ethanolamine for 4 h. The hot ethanolamine solution was then poured into 50 mL of cold water, and the aqueous solution was extracted four times with 25 mL of $CH_2Cl_2$. The combined organic layers were thoroughly washed with water in order to remove any trace of ethanolamine, and then dried over magnesium sulfate.

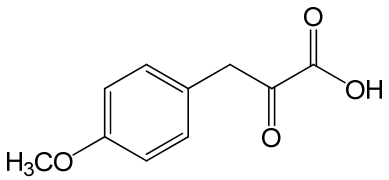
#### 5.2.1.12 2-Methyl-4-[4-(methoxy)benzylidene]-5(4*H*)-oxazolone (22)




A mixture of *p*-anisaldehyde **(21)** (13.7 ml, 113 mmol), N- acetyl-glycine (15.87 g, 135.6 mmol) and sodium acetate (12.05 g, 146.9 mmol) in acetic anhydride (14 ml, 146.9 mmol) was stirred at reflux for 1 h. The reaction was quenched with ice (approx. 50 mL) and vigorously stirred for 1 h in an ice bath to allow precipitation. Filtration of the solid afforded the title compound **(22)** (17.18 g, 70%) as a yellow solid. mp: 174 – 177 °C; IR (Neat) $\nu_{max}$ 1770, 1658, 1597, 1254 $cm^{-1}$; $^1H$ NMR (DMSO): δ 2.35 (s, 3H), 3.82 (s, 3H), 7.05 (d, 2H, *J* = 8.85), 7.16 (s, 1H), 8.14 (d, 2H, *J* = 8.85). $^{13}C$ NMR

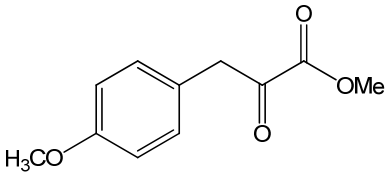
(DMSO): δ 15.33, 55.52, 114.61 (2C), 125.87, 130.19, 134.19 (2CH,1C), 161.72, 165.48, 167.66.

### 5.2.1.13 3-(4-Methoxyphenyl)-2-oxopropionic acid [(4-methoxyphenyl) pyruvic acid] (23)




A suspension of **(31)** (10 g, 46.04 mmol) in aq. HCl (3 M, 100 mL) was stirred at reflux for 3 h. The mixture was cooled to RT to allow crystallization, and then filtered to afford the title compound **(23)** (8.94 g, 95%) as an orange solid. IR (Neat) $\nu_{max}$ 3440-2935 (OH), 1719, 1602, 1509, 1243, 1169, 1024 $cm^{-1}$; $^{1}H$ NMR (DMSO): δ 3.74 (s, 3H), 6.36 (s, 1H), 6.9 (d, 2H, *J* = 8.85), 7.69 (d, 2H, *J* = 8.85), 8.95 (br s, 1H). $^{13}C$ NMR (DMSO): δ 20.89, 109.71, 128.92, 129.22, 132.1, 136.57, 141.10, 166.38.

### 5.2.1.14 Methyl 3-(4-methoxyphenyl)-2-oxopropanoate [3-(4-hydroxyphenyl) pyruvat] (24)



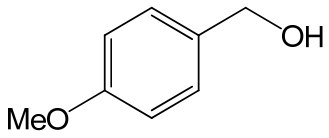

To a solution of (4-methoxyphenyl) pyruvic acid **(23)** (4.44 g, 22.88 mmol) in DMF (150 ml) at 0 ºC was added DBU (3.42 ml, 22.88 mmol) and MeI (5.7 ml, 91.52mmol). The mixture was stirred for 4.5 h at the same temperature, acidified with 1 M HCl and extracted with diethyl ether (2 x 50 ml). The combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated to dryness to give the title compound **(24)** (4.29 g, 90%) as yellow oil. $^{1}H$ NMR (DMSO): δ 3.74 (s, 3H), 3.76 (s, 3H), 6.39 (s, 1H),

6.91 (d, 2H, *J* = 8.25), 7.71 (d, 2H, *J* = 8.25), 9.26 (s, 1H).

#### 5.2.1.15 General procedure for the coupling reaction of arylpyrovic ester with ammonia

To a solution of methyl 3-(4-hydroxyphenyl) pyruvate **(24)** (0.65 g, 3.32 mmol) in anhydrous MeOH (120 ml) was added NaOMe (1.62 g, 30 mmol) under $N_2$ atmosphere. The mixture was stirred for 15 min and then treated dropwise with a solution of $I_2$ (0.42 g, 1.66 mmol) in MeOH (100 mL). After stirring at room temperature for 45 min., concd aq ammonia (1 ml) was added, and the stirring continued for 30 min. The reaction was completed by heating the solution to 60 °C in a sealed tube for 1h followed by refluxing for 4 h. After reducing the volume to 100 ml by evaporation, 15 ml of THF was added, followed by the addition of LiOH (0.5 g, 12 mmol) and refluxed overnight. The reaction mixture was diluted with water and extracted with diethyl ether to remove any traces of starting materials. The water layer was separated and acidified with HCl (1N) to pH 2 and extracted with $CHCl_3$ three times. The organic layer was dried over sodium sulfate and evaporated to give white needles which were used in the following step without any further purification. The resultant acid was refluxed under nitrogen in 25 mL of freshly distilled ethanolamine for 4 h. The hot ethanolamine solution was then poured into 50 mL of cold water, and the aqueous solution was extracted four times with 25 mL of $CH_2Cl_2$. The combined organic layers were thoroughly washed with water in order to remove any trace of ethanolamine, and then dried over magnesium sulfate.

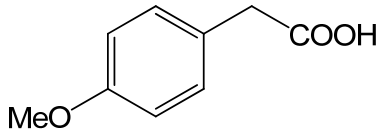
#### 5.2.1.16 (4-methoxyphenyl) methanol (38)




To a solution of *p*-anisaldehyde **(21)** (6 g, 44.07 mmol) in a mixture of THF (24 ml) and $H_2O$ (36 ml) was added $NaBH_4$ (1.68 g, 44.07 mmol) portionwise and the reaction

mixture was stirred for 0.5 h at room temperature. The reaction mixture was extracted with EtOAc (50 ml) and the organic layer was washed successively with (1N) HCl (50 ml) and brine. Evaporation of the solvent under reduced pressure afforded **(38)** (5.79 g, 95%) as clear liquid. IR (Neat) $\nu_{max}$ 3322, 2935, 2835, 1609, 1511, 1242, 1028, 813 $cm^{-1}$; $^1H$ NMR (DMSO): δ 3.72 (s, 3H), 4.41 (d, 2H, J = 5.5), 5.06 (t, 1H), 6.87 (d, 2H, *J* = 8.55), 7.22 (d, 2H, *J* = 8.55). $^{13}C$ NMR (DMSO): δ 55.01, 62.59, 113.46, 127.95, 134.53, 158.18.

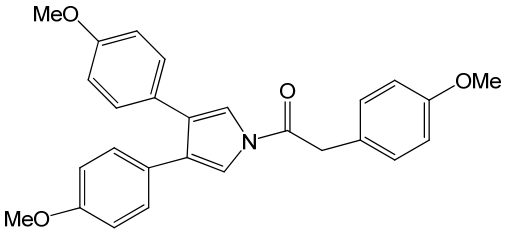
### 5.2.1.17 4-methoxyphenylacetic acid (41)




To a solution of (4-methoxyphenyl) methanol **(38)** (5.79 g, 41.91 mmol) in dichloromethane (60 ml) at 0°C was added thionyl chloride (7.07 ml, 96.95 mmol) dropwise. The mixture was stirred overnight at room temperature, and washed with water (50 ml) and dried over sodium sulfate. The solvent was evaporated under reduced pressure at 30 °C to afford corresponding benzyl chloride as a clear liquid, which was used in the next step without any further purification. After dissolving the chloride in DMF (60 ml) NaCN (2.37 g, 48.47 mmol) was added, and the reaction was stirred overnight at room temperature. The reaction mixture was extracted in ethyl acetate (70 ml) and washed with $H_2O$ (30 ml), (1N) HCl (30 ml) and brine. After drying over sodium sulfate, the solvent was removed under reduced pressure to obtain the cyanide derivative, which was dissolved in methanol (40 ml) and treated with (4 N) NaOH (40 ml). The reaction mixture was refluxed overnight, and extracted with diethyl ether to remove any traces of the starting materials. The water layer was acidified with (1N) HCl to pH 2, and the solid formed was filtered and then washed with $H_2O$ to afford the title compound **(41)** (5.13 g, 70 %) as white crystals. mp: 78 – 80 °C. IR (Neat) $\nu_{max}$ 2942, 2739, 1692, 1412, 1236, 1021 $cm^{-1}$. $^1H$ NMR (DMSO): δ 3.47 (s, 2H), 3.71 (s, 3H),

6.85 (d, 2H, J = 8.55), 7.15 (d, 2H, J = 8.55), 12.24 (br s, 1H).$^{13}$C NMR (DMSO): 39.80, 55.03, 113.68, 126.96, 130.39, 158.04, 173.03.

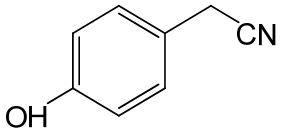
### 5.2.1.18 1-(3,4-bis(4-methoxyphenyl)-1*H*-pyrrol-1-yl)-2-(4-methoxyphenyl) ethanone (44)




To a solution of 4-methoxyphenylacetic acid **(41)** (65.2 mg, 0.39 mmol) in dry dichloromethane (4 mL) under $N_2$ atmosphere at 0 °C was added oxalyl chloride (58.8 mg, 0.46 mmol) dropwise, followed by the addition of 2 drops of DMF. The reaction mixture was stirred at the same temperature for 15 min, and then at room temperature for 2 h, and evaporated to dryness. In another flask, a solution of 3,4-Bis(4-methoxyphenyl)-1*H*-pyrrole **(20)** (54.6 mg, 0.19 mmol) in DMF (5 ml) was cooled to 0 - 5 °C, followed by the portionwise addition of NaH (11.8 mg, 0.49 mmol) and 0.5 g of oven-dried molecular sieves. After being stirred for 0.5 h at room temperature, the acid chloride solution **(41)** in THF (0.5 ml) at 0 °C was added dropwise to the reaction mixture over the course of 15 min. The reaction mixture was stirred overnight at room temperature, then diluted with ethyl acetate and washed successively with sat. $NaHCO_3$ and brine. The organic layer was dried over sodium sulfate and purified by column chromatography eluting with ethyl acetate:hexanes (1:5), to afford the title compound **(44)** (32.49 mg, 40%) as yellow liquid. IR (Neat) $\nu_{max}$ 3347, 2959, 1669, 1602, 1510, 1250, 1024, 792 cm$^{-1}$; $^1$H NMR ($CDCl_3$): δ 3.8 (s, 9H), 4.12 (s, 2H), 6.82 (d, 4H, *J* = 8.85), 6.90 (d, 2H, *J* = 8.85), 7.15 (d, 4H, *J* = 8.85), 7.19 (d, 2H, *J* = 8.85), 7.39 (br s, 2H).$^{13}$C NMR ($CDCl_3$): 40.54, 55.23, 55.30, 113.63, 113.76, 114.38, 116.74, 117.21, 125.07, 126.36, 129.60, 129.65, 130.23, 158.68. MS (m/z): 427 ($M^+$), 295, 135, 77.

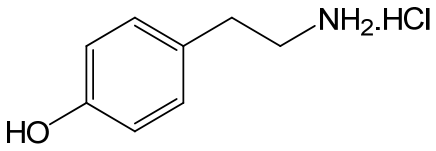
## 5.3 Synthesis of Aplysamine 6

### 5.3.1.1 2-(4-hydroxyphenyl) acetonitrile (58)




To a solution of 4-(hydroxymethyl) phenol **(57)** (4 g, 32.22 mmol) in DMF (100 ml) was added NaCN (1.89 g, 38.66 mmol). The mixture was heated at 130 °C for 24 h. The reaction mixture was extracted in ethyl acetate (150 ml) and washed with $H_2O$, (1N) HCl (Caution-HCN), $H_2O$, and brine. After drying the organic layer over anhydrous sodium sulfate, the solvent was removed under reduced pressure to get **(58) (**2.79 g, 65%) as white solid. $^1$H NMR ($CDCl_3$): δ 3.68 (s, 2H), 5.61 (br s, 1H), 6.84 (d, 2H, *J* = 8.55), 7.18 (d, 2H, *J* = 8.55). $^{13}$C NMR ($CDCl_3$): δ 22.81, 116 (2CH), 118.23, 121.59, 129.24 (2CH), 155.61.

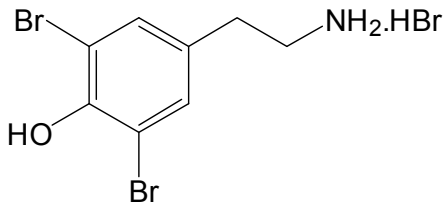
### 5.3.1.2 4-hydroxyphenethylamine (59)




To a solution of **(58)** (2.79 g, 20.95 mmol) in a mixture of EtOH (30 ml) and (3N) methanolic-HCl (14 ml) in a Parr pressure vessel was added 10% palladium on charcoal (0.4 g). The mixture was subjected to hydrogenation at 50 psi for 12 h. The reaction mixture was filtered through a pad of Celite® and rinsed thoroughly with ethanol. Removal of the solvent under reduced pressure gave pure **(59)** (3.46 g, 95%) as an off-white solid; $^1$H NMR (DMSO): δ 2.76 (t, 2H), 2.91 (m, 2H), 6.71 (d, 2H, J = 8.25), 7.01

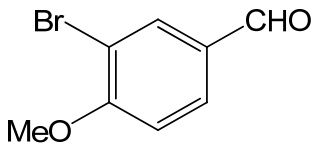
(d, 2H, J = 8.25), 8.13 (s, 3H), 9.39 (s, 1H). $^{13}$C NMR (DMSO): δ 32.11, 40.22, 115.35, 127.26, 129.49, 156.15.

#### 5.3.1.3 4-(2-aminoethyl)-2,6-dibromophenol hydrobromide (60)




To a solution of 4-hydroxyphenethylamine **(59)** (3.40 g, 19.58 mmol) in methanol (50 ml) was added $Br_2$ (2.21 ml, 43.08 mmol). The reaction mixture was stirred at 60°C for 12 h. The solvent was removed under reduced pressure to obtain the title compound **(60)** (7.21 g, 98 %) as an off white solid. $^1$H NMR (DMSO): δ 2.75 (t, 2H), 3.01 (t, 2H), 7.45 (s, 2H), 7.76 (s, 3H), 9.95 (br s, 1H). $^{13}$C NMR (DMSO): δ 31.39, 39.90, 112.11, 131.78, 132.75 (2CH), 149.72.

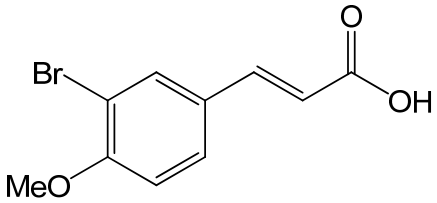
#### 5.3.1.4 3-bromo-4-methoxybenzaldehyde (47)




To a solution of *p*-anisaldehyde **(21)** (2 g, 14.69 mmol) in dichloromethane (25 ml) was added $Br_2$ (0.9 ml, 17.63 mmol) dropwise. The reaction mixture was stirred at 60 °C for 24 h. The mixture was diluted with $CH_2Cl_2$ (30 ml) and washed with water (20 ml), sat. $NaHCO_3$ (20 ml) and brine (20 ml). The organic layer was dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure to get **(47)** (2.53 g, 80 %) as white solid. $^1$H NMR (DMSO): δ 3.94 (s, 3H), 7.28 (d, 1H, *J* = 8.55), 7.90

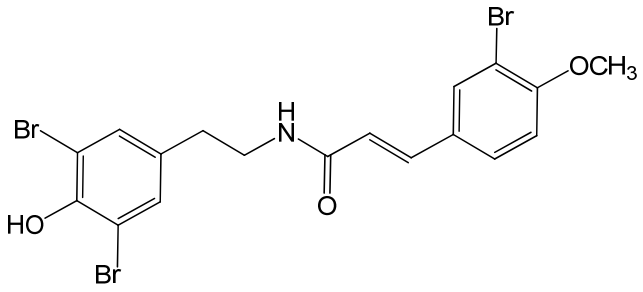
(dd, 1H, *J* = 8.55, 2.1), 8.06 (d, 1H, J = 2.1), 9.83 (s, 1H). $^{13}$C NMR (DMSO): δ 57.07, 111.57, 112.99, 130.69, 131.54, 133.98, 160.25, 190.78.

### 5.3.1.5 (3-bromo-4-methoxyphenyl) acrylic acid (46)



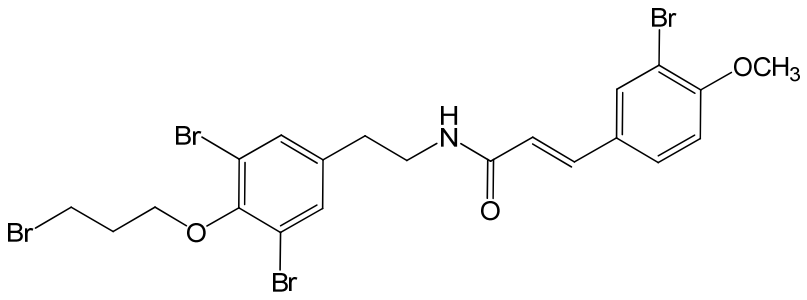

To a solution of 3-bromo-4-methoxybenzaldehyde **(47)** (1.86 g, 8.63 mmol) in a mixture of pyridine (20 ml) and piperidine (2.84 ml, 28.78 mmol) was added malonic acid (0.65 g, 7.19 mmol). The reaction mixture was heated at 120 °C overnight. After cooling to ambient temperature, $H_2O$ (15 ml) was added, followed by the addition of (1N) NaOH to make pH 10. The mixture was extracted with diethyl ether (20 ml × 3) and the aqueous layer was acidified with 2N HCl to pH 2 and extracted with ethyl acetate (25 ml × 2). The combined organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure to give **(46)** (2.04 g, 92 %) as light yellow solid. $^{1}$H NMR (DMSO): δ 3.87 (s, 3H), 6.44 (d, 1H, J = 15.85), 7.12 (d, 1H, J = 8.55), 7.49 (d, 1H, H = 15.85), 7.68 (dd, 1H, *J* = 8.55, 2.45), 7.94 (d, 1H, *J* = 2.45). $^{13}$C NMR (DMSO): δ 56.62, 111.34, 112.93, 118.20, 128.52, 129.55, 132.65, 142.52, 156.93, 167.83.

### 5.3.1.6 (E)-3-(3-bromo-4-methoxyphenyl)-N-(3,5-dibromo-4-hydroxyphenethyl) acrylamide (62)




To a solution of cinnamic acid **(46)** (0.3 g, 1.17 mmol) in dry THF (10 mL) at 0 °C was added thionyl chloride (0.42 g, 3.51 mmol) dropwise, and the reaction was stirred overnight at room temperature. The solvent was evaporated under reduced pressure, and the acid chloride **(61)** was kept under high vacuum for 1 h. In another flask, a solution of amine compound **(60)** (0.45 g, 1.29 mmol) in a mixture of dry THF (15 mL) and DMF (15 mL) was cooled to between 5 and 10 °C, followed by the dropwise addition of 2,6-lutidine (0.55 g, 5.16 mmol). After being stirred for 0.5 h, a solution of **(61)** in dry THF (6 mL) was added dropwise to the reaction mixture over the course of 15 min. The reaction mixture was stirred for 1 h at the same temperature. Then it was diluted with ethyl acetate and washed successively with 2 N HCl, brine, sat. $NaHCO_3$, and brine and dried over sodium sulfate. Column chromatography of the dark orange oily material, eluted with ethyl acetate:hexanes (4:6), afforded amide **(62)** (0.43 g, 70%) as a white crystalline solid. mp: 172–173 °C. Anal. Calcd for $C_{18}H_{16}Br_3NO_3$ requires C, 40.48; H, 3.02; N, 2.62. Found: C, 40.42; H, 3.06; N, 2.59; IR (KBr) $\nu_{max}$ 3415, 3250, 1675, 1530, 1485, 1245, 1150 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$; 500 MHz): δ 2.66 (t, 2H, *J* = 7), 3.36 ($CH_2NHCO$-; obscured by the DMSO signal), 3.86 (s, 3H), 6.50 (d, 1H, *J* = 15.8), 7.13 (d, 1H, *J* = 8.5 Hz), 7.30 (d, 1H, *J* = 15.8), 7.39 (s, 2H), 7.54 (dd, 1H, *J* = 8.5, 2.1 Hz), 7.77 (d, 1H, *J* = 2.1), 8.02 (t; 1H). $^{13}C$ NMR (DMSO-$d_6$; 125.7 MHz): δ 33.3 ($CH_2$), 39.6 ($CH_2$; obscured by DMSO peak), 56.3 ($OCH_3$), 111.0 (C), 112.8 (CH), 119.2 (2C), 121.2 (CH), 128.4 (CH), 129.1 (C), 131.6 (CH), 131.5 (C), 132.2 (2CH), 136.7 (CH), 149.0 (C), 156.1 (C), 164.9 (C).

### 5.3.1.7 Aplysamine 6 (11)




To a solution of **(62)** (0.4 g, 0.75 mmol) in DMF (10 ml) was added sodium carbonate (0.2 g, 1.87 mmol) followed by the dropwise addition of 1,3-dibromopropane (0.23 g, 1.13 mmol). The reaction mixture was stirred at room temperature for 20 h. Water was added to the reaction, and the mixture extracted with ethyl acetate (50 ml). The organic layer was washed with 1N HCl and brine, and then dried over anhydrous sodium sulfate. Solvent was removed under reduced pressure to get the alkylated product as light yellow thick oil, which was purified by column chromatography eluting with EtOAc : Hex (2:4) and changing to (3:7) to get the title compound as colorless thick oil. The bromide intermediate was dissolved in THF (10 ml) and treated with an excess of $NH_4OH$ (5 ml). The reaction was stirred in sealed tube at 80 $^{\circ}$C for 8 h. Water was added and the mixture was extracted with ethyl acetate, and the organic layer was washed with (1N) HCl and brine. After drying over anhydrous sodium sulfate the product was purified by column chromatography using hexane/ethyl acetate (5: 1 to 4 : 1) as eluent to give pure **(11)** (0.39 g, 88 %) as colorless gum. Anal. Calcd for $C_{21}H_{23}Br_3N_2O_3$ requires C, 42.67; H, 3.92; N, 4.74. Found: C, 42.59; H, 3.98; N, 4.66. IR (KBr) $\nu_{max}$ 3410, 3273, 3061, 1682, 1540, 1497, 1458, 1260, 1201, 1138, 1050 $cm^{-1}$. $^1$H NMR (DMSO-$d_6$; 500 MHz): δ 1.92 (m, 2H), 2.60 (t, 2H, *J* = 6.4 Hz), 2.72 (m, 2H, *J* = 6.4 Hz), 3.39 (m, 2H, J = 6.4 Hz), 3.85 (s, 3H), 3.96 (t, 2H, *J* = 6.5 Hz), 6.50 (d, 1H, *J* = 15.6 Hz), 7.12 (d, 1H, *J* = 8.5 Hz), 7.32 (d, 1H, *J* = 15.6 Hz), 7.49 (s, 2H), 7.53 (dd, 1H, *J* = 8.5, 1.8 Hz), 7.76 (d, 1H; *J* = 1.8 Hz), 8.05 (t, 1H). $^{13}$C NMR (DMSO-$d_6$; 125.7 MHz): δ 28.9 ($CH_2$), 33.5 ($CH_2$), 39.6 ($CH_2$; obscured by the DMSO signal), 45.5 ($CH_2$),

56.4 ($OCH_3$), 71.4 ($CH_2$), 111.1 (C), 112.9 (CH), 117.3 (2C), 121.1 (CH), 128.5 (CH), 129.0 (C), 131.6 (CH), 132.9 (2 CH), 136.9 (CH), 139.0 (C), 150.7 (C), 156.1 (C), 165.0 (C).

# CHAPTER 6

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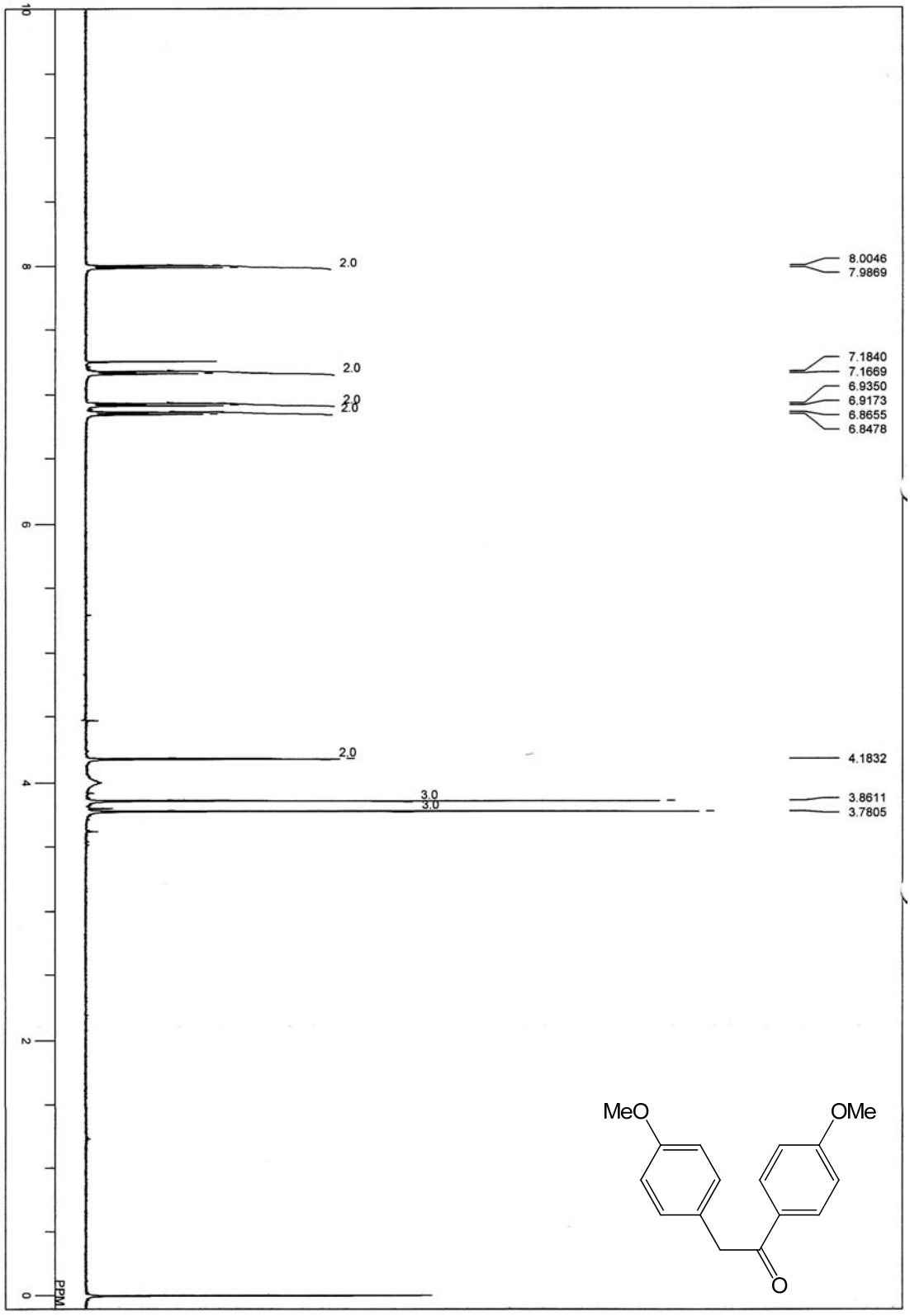
# APPENDIX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OMe
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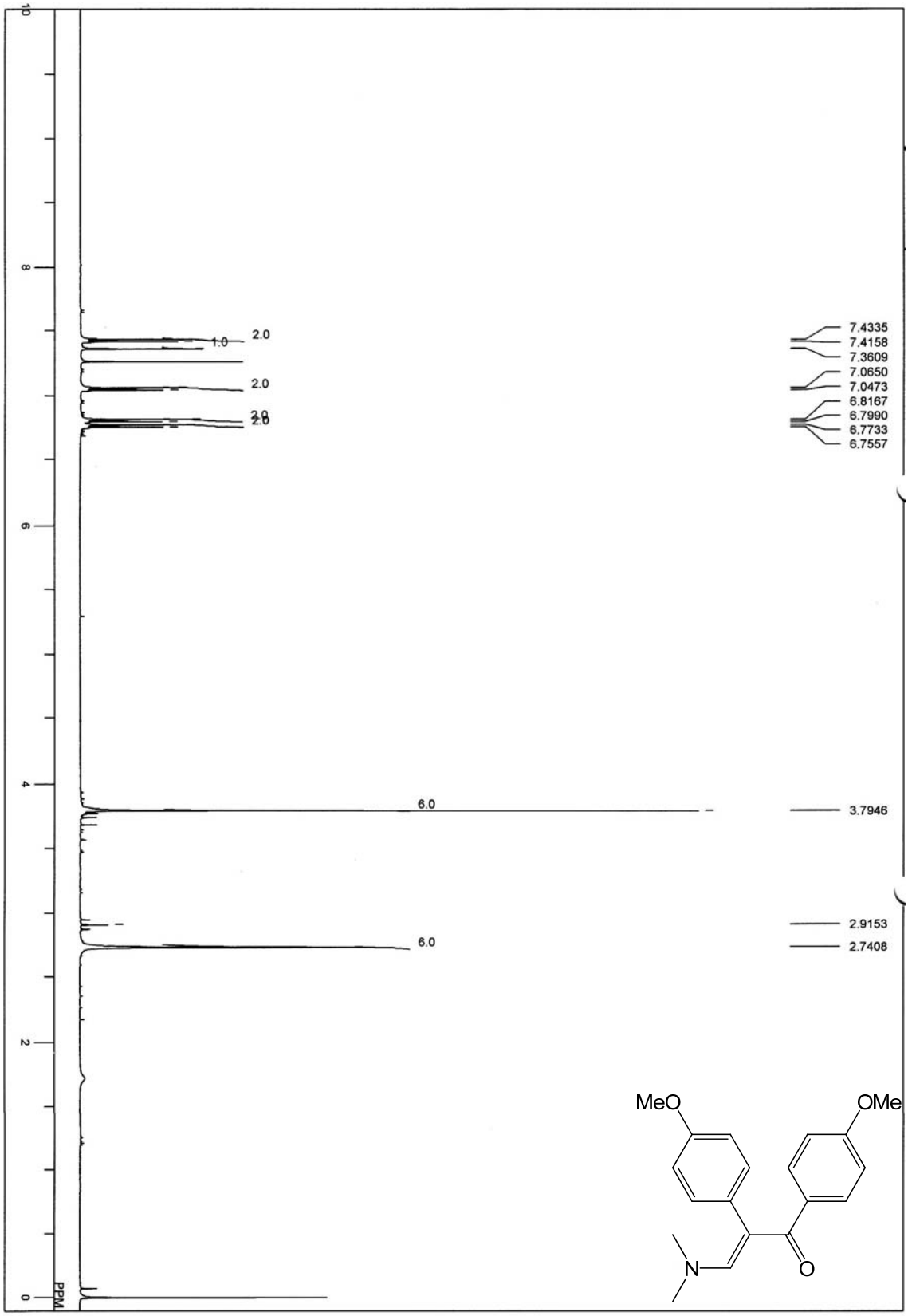
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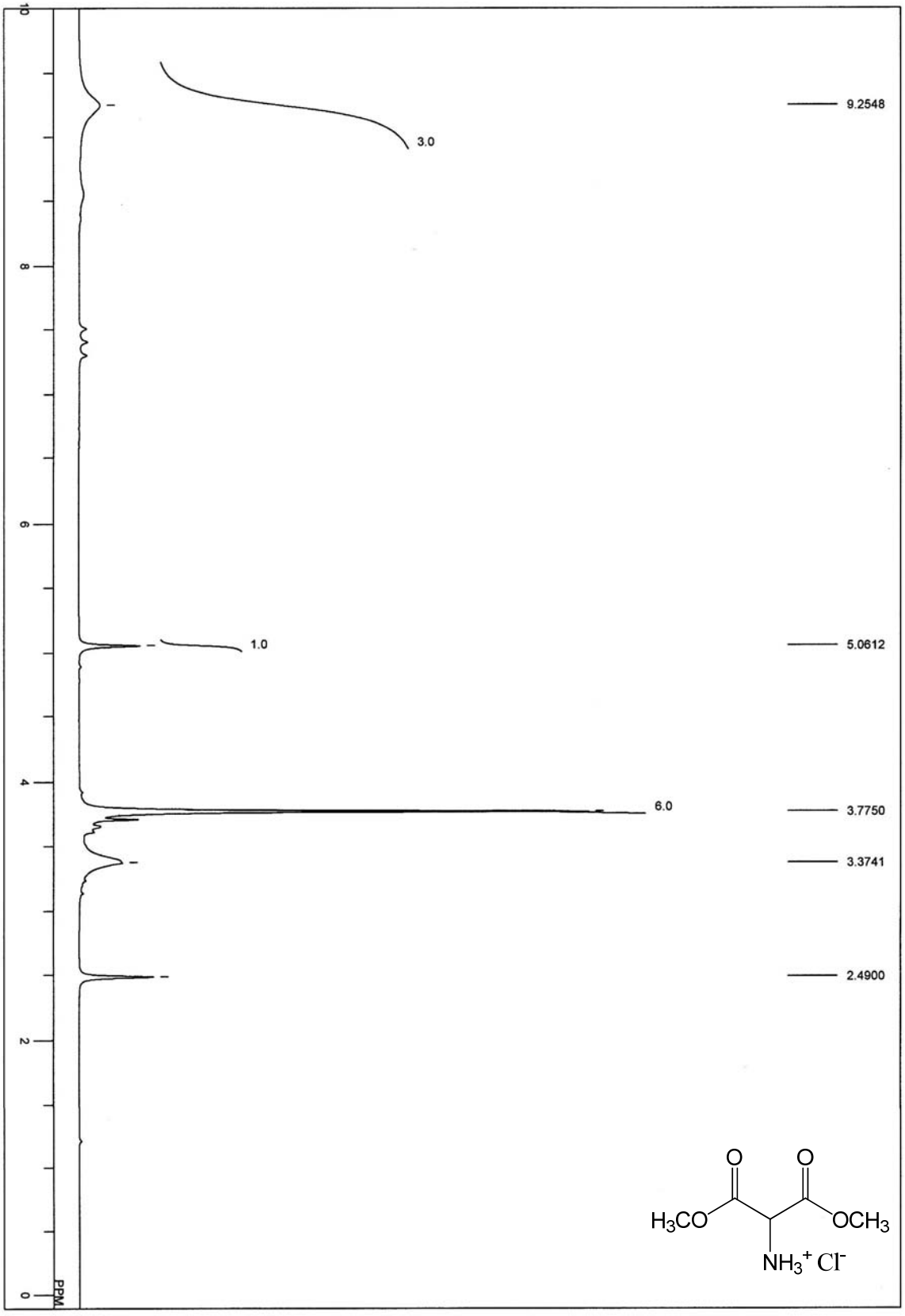
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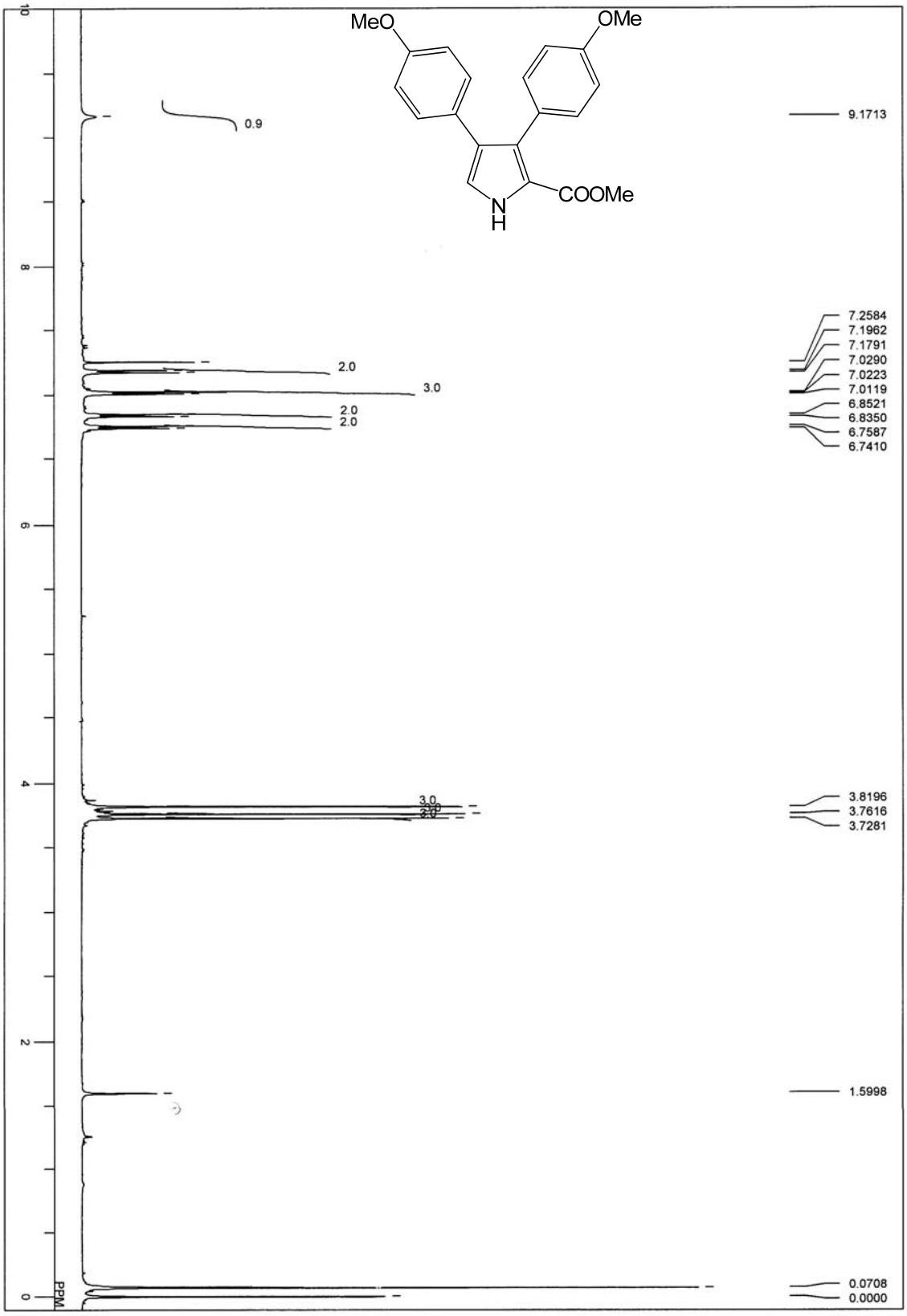
OCH3
NH3+ Cl-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7.1791
7.0290
7.0223
7.0119
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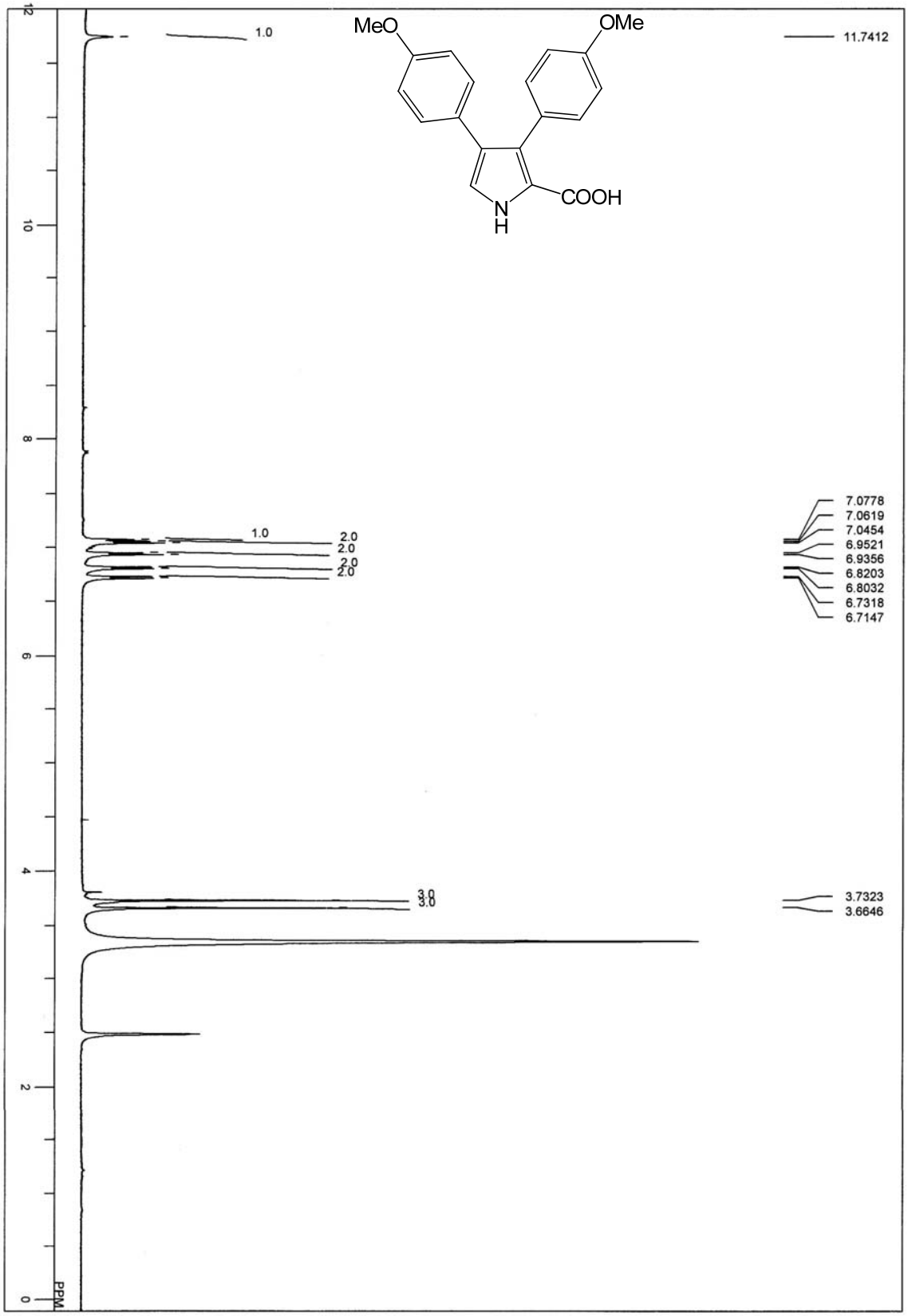
PPM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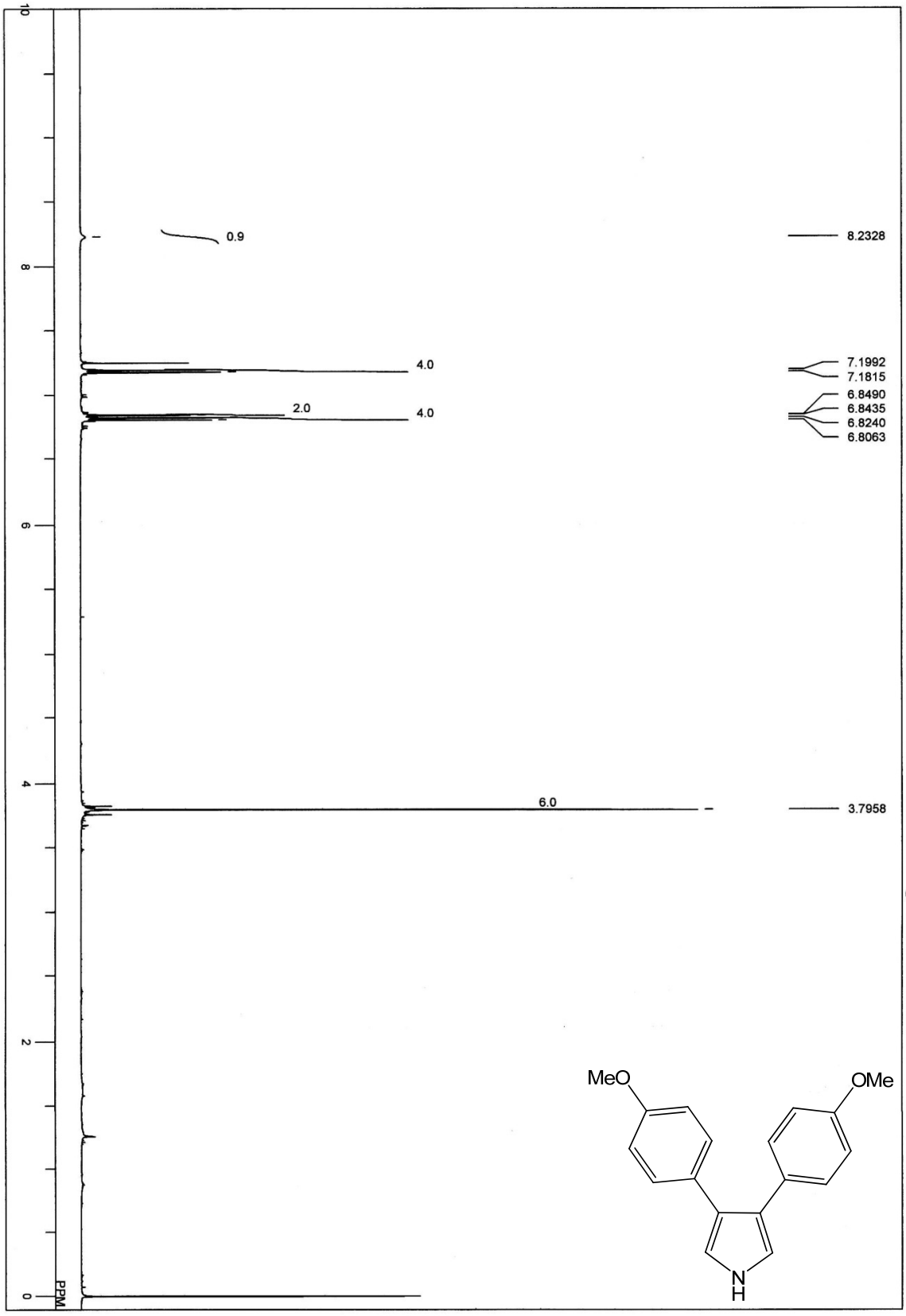
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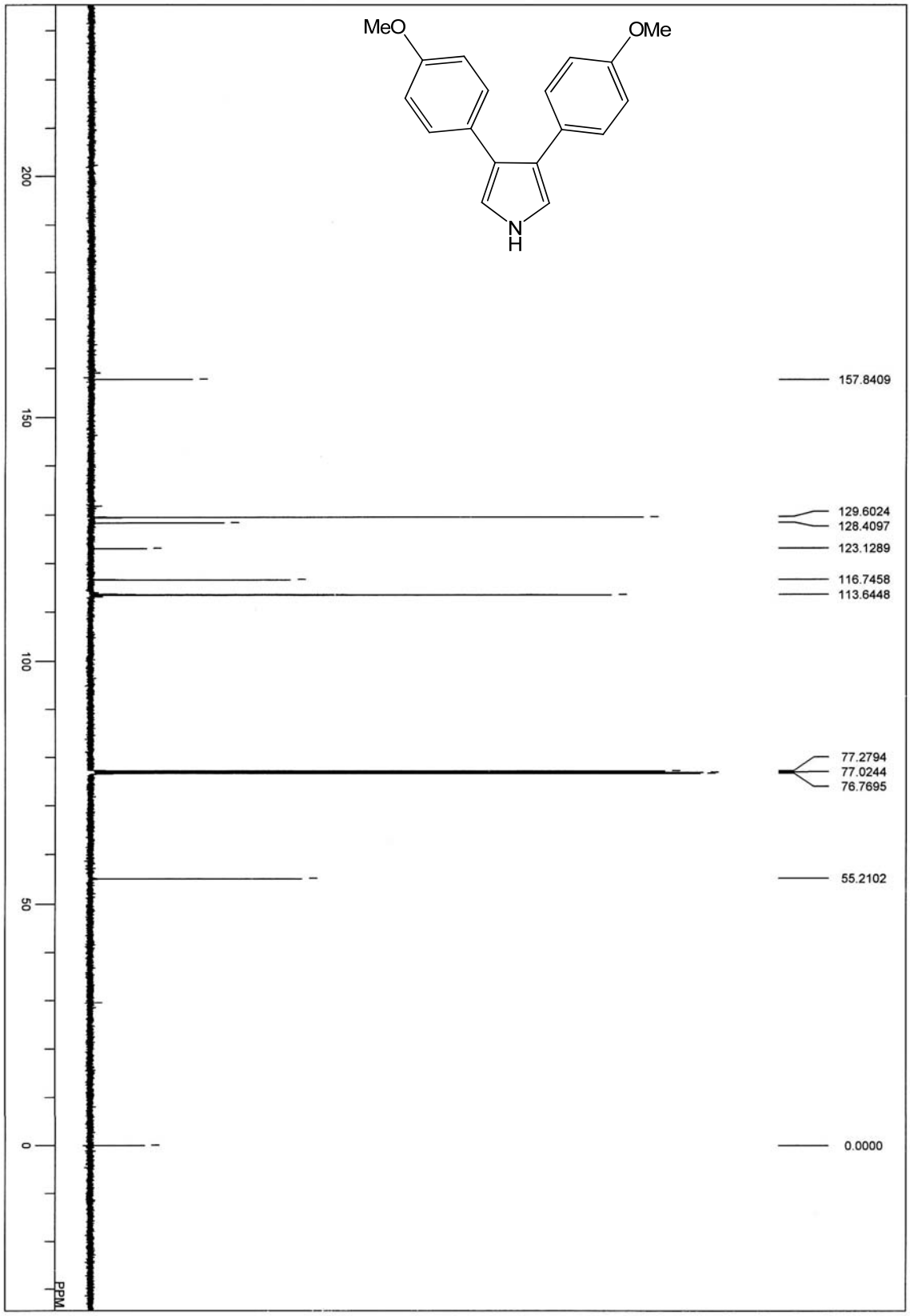
N
H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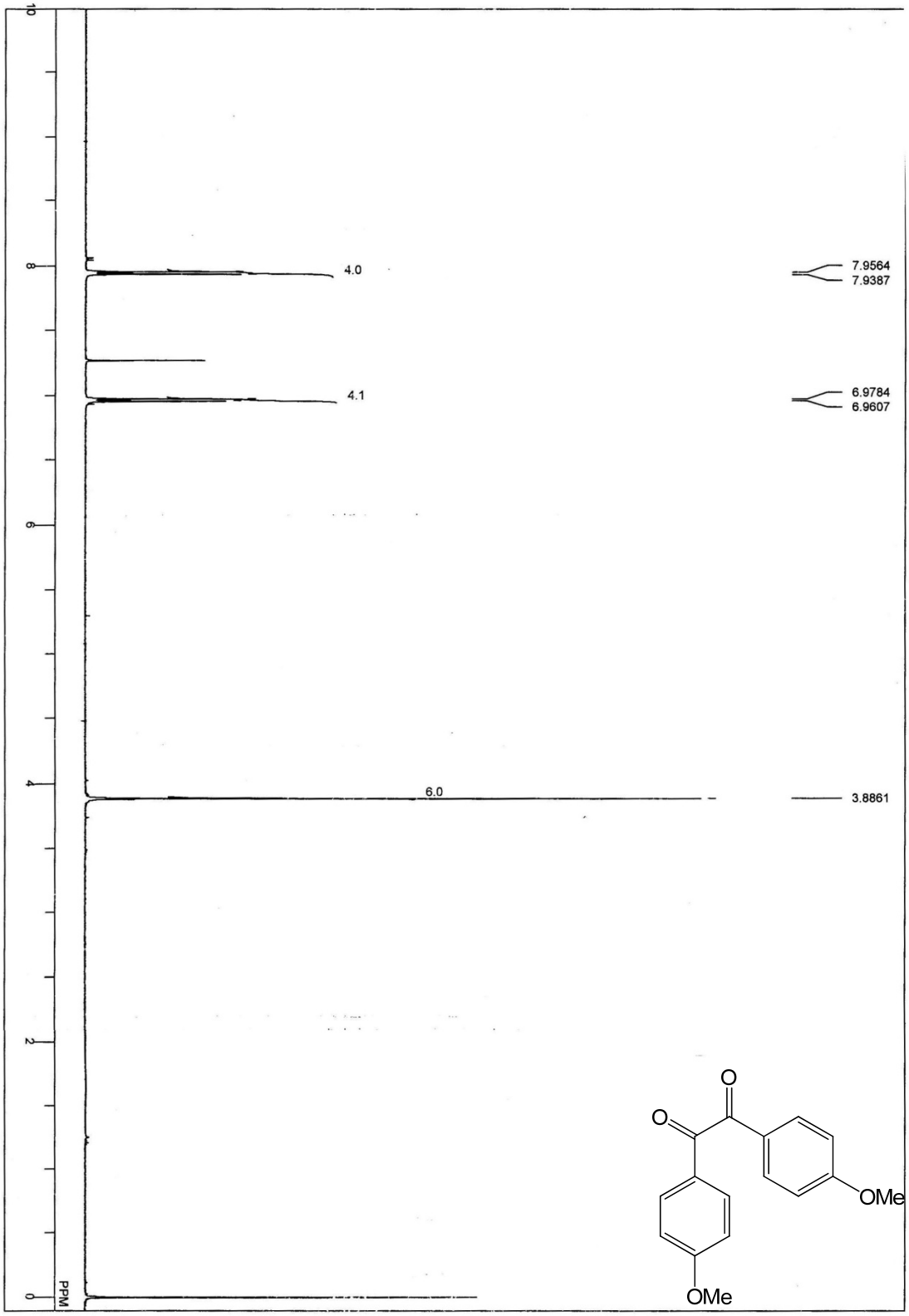
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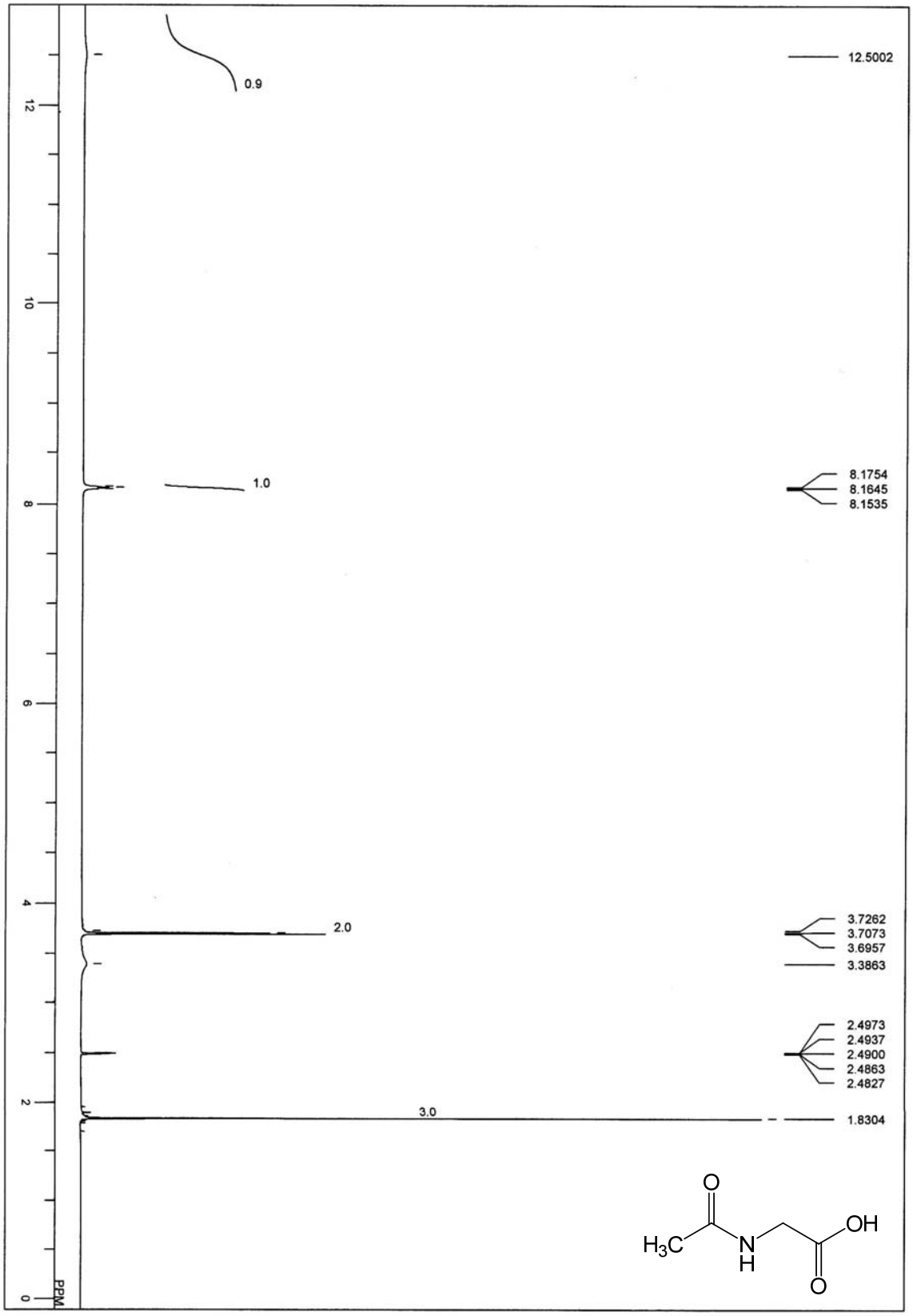
OMe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H
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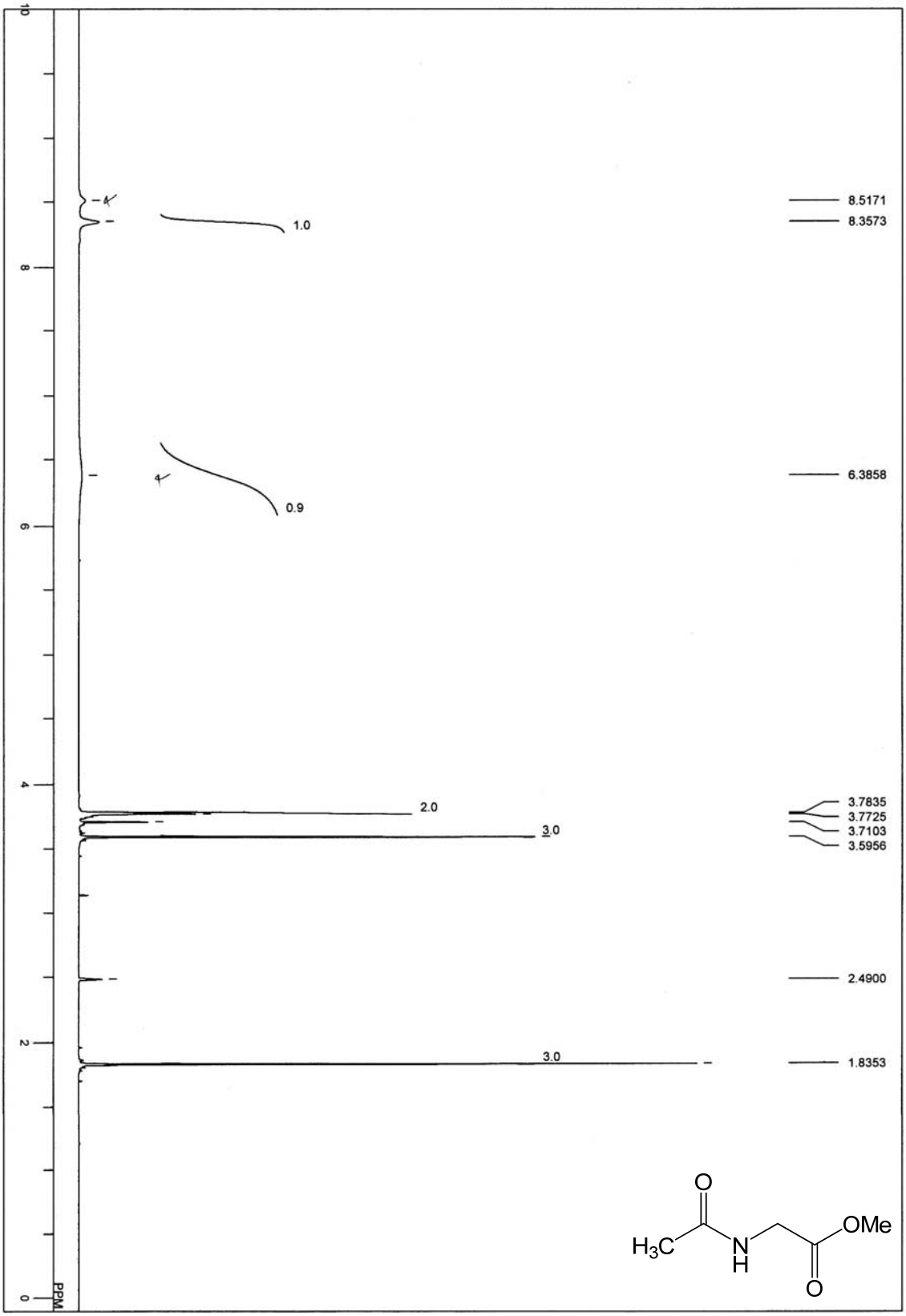
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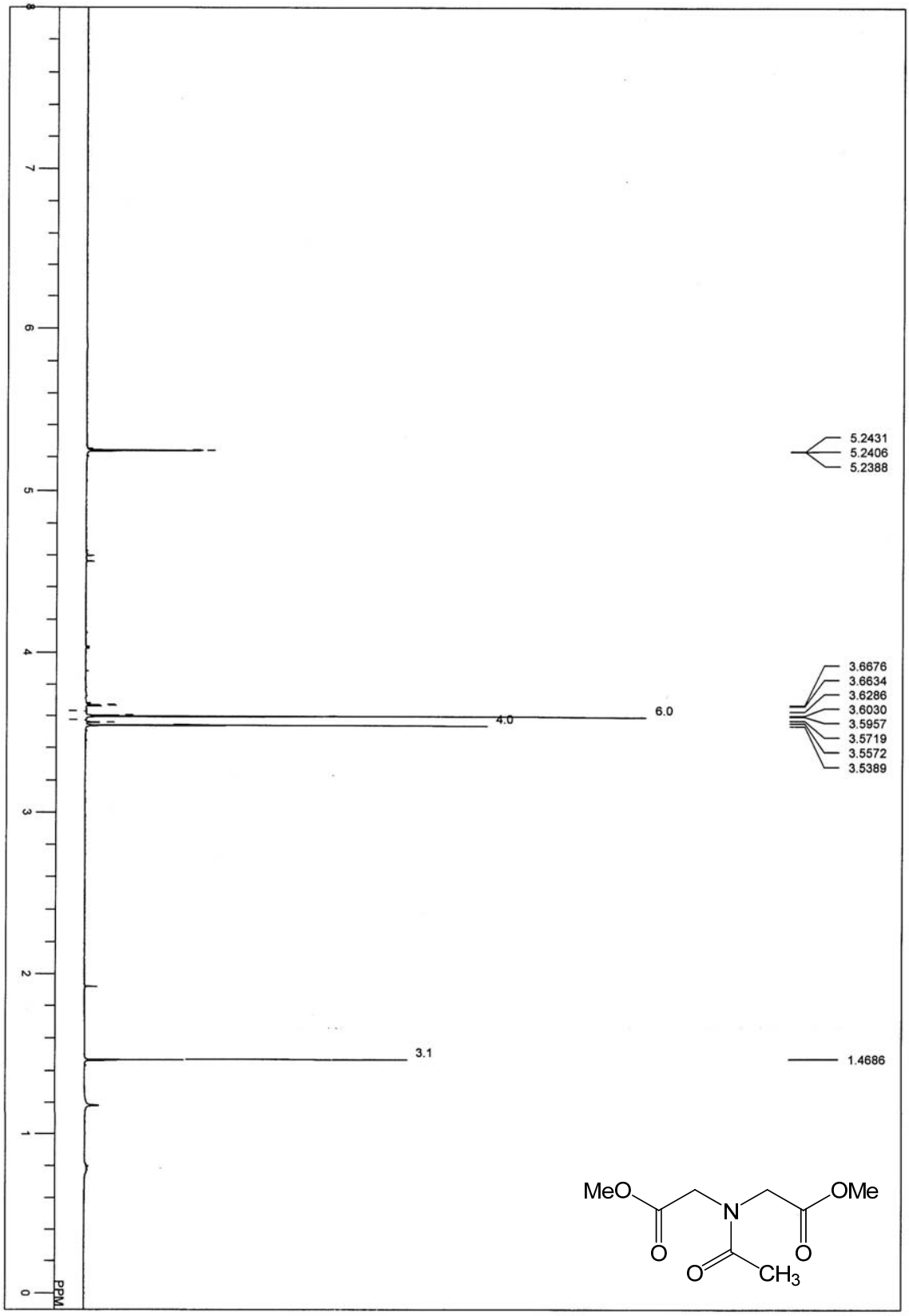
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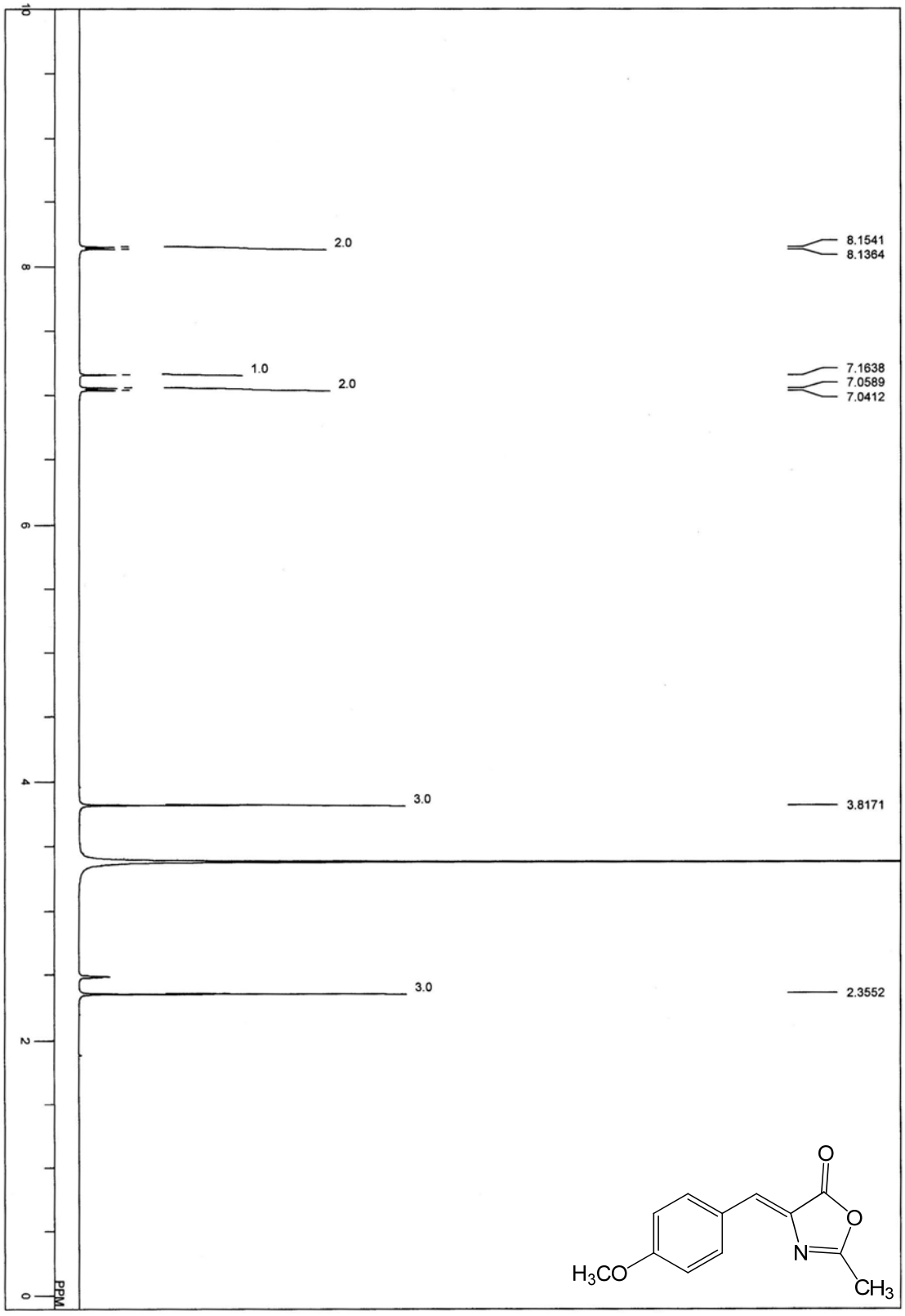
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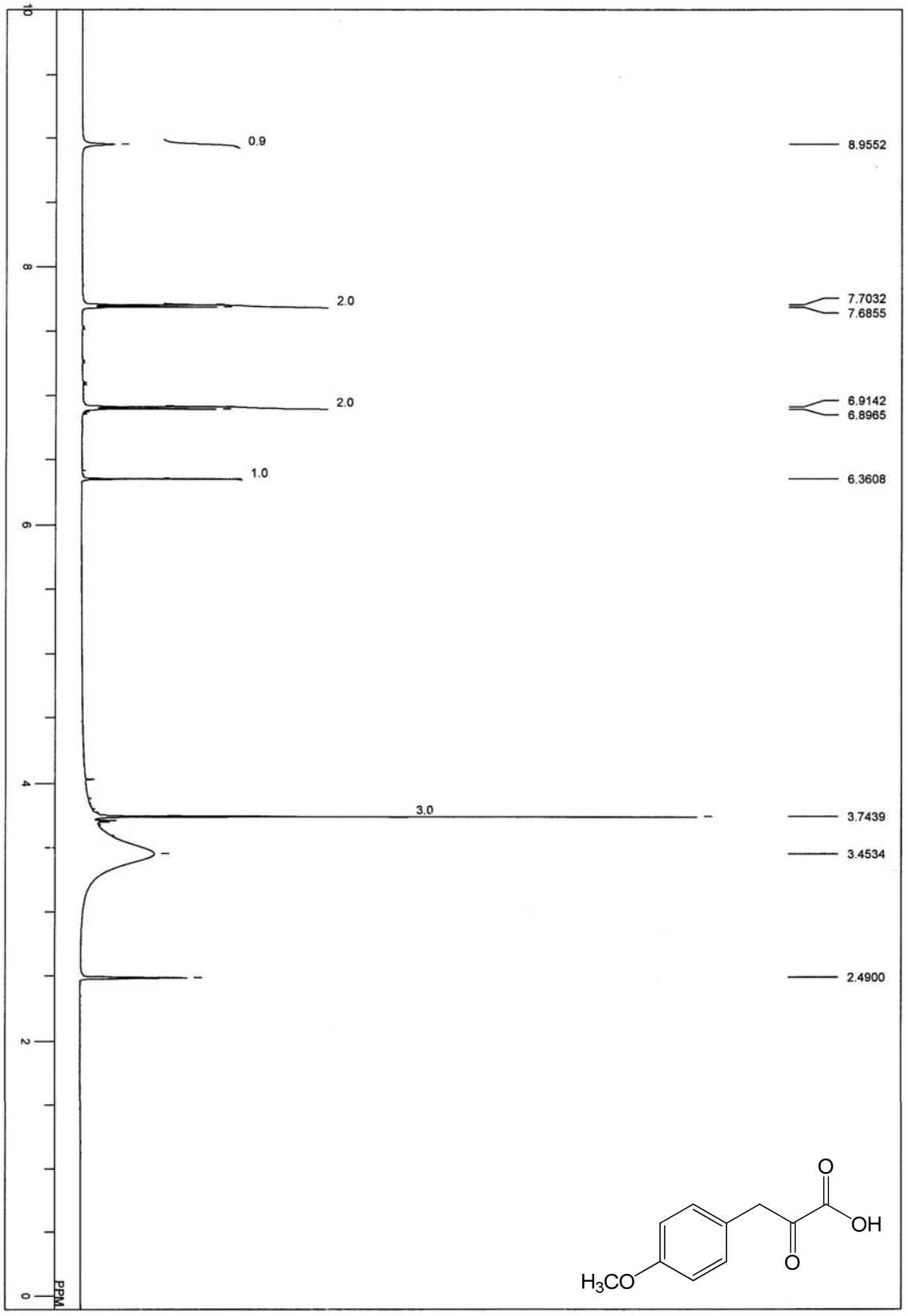
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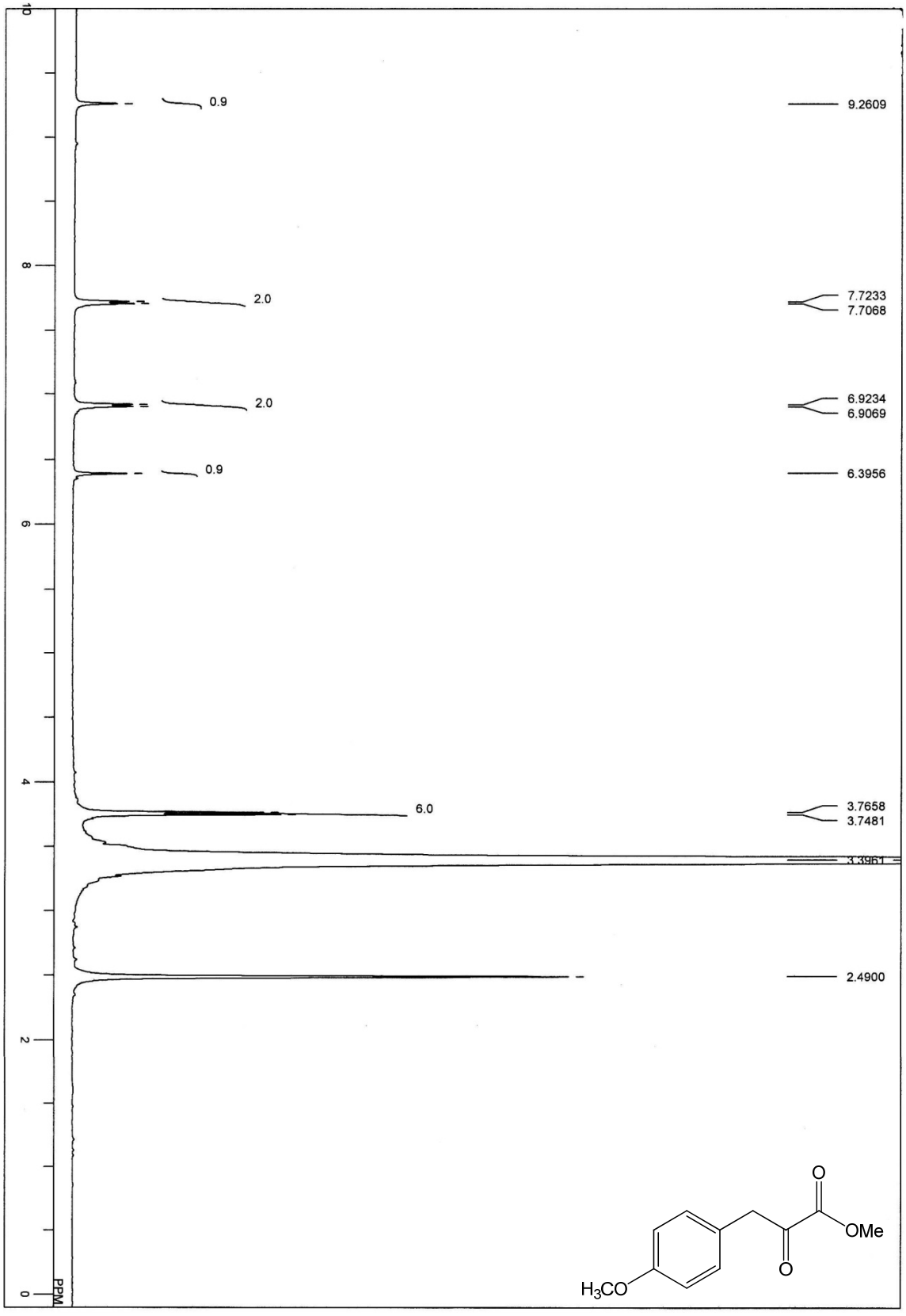
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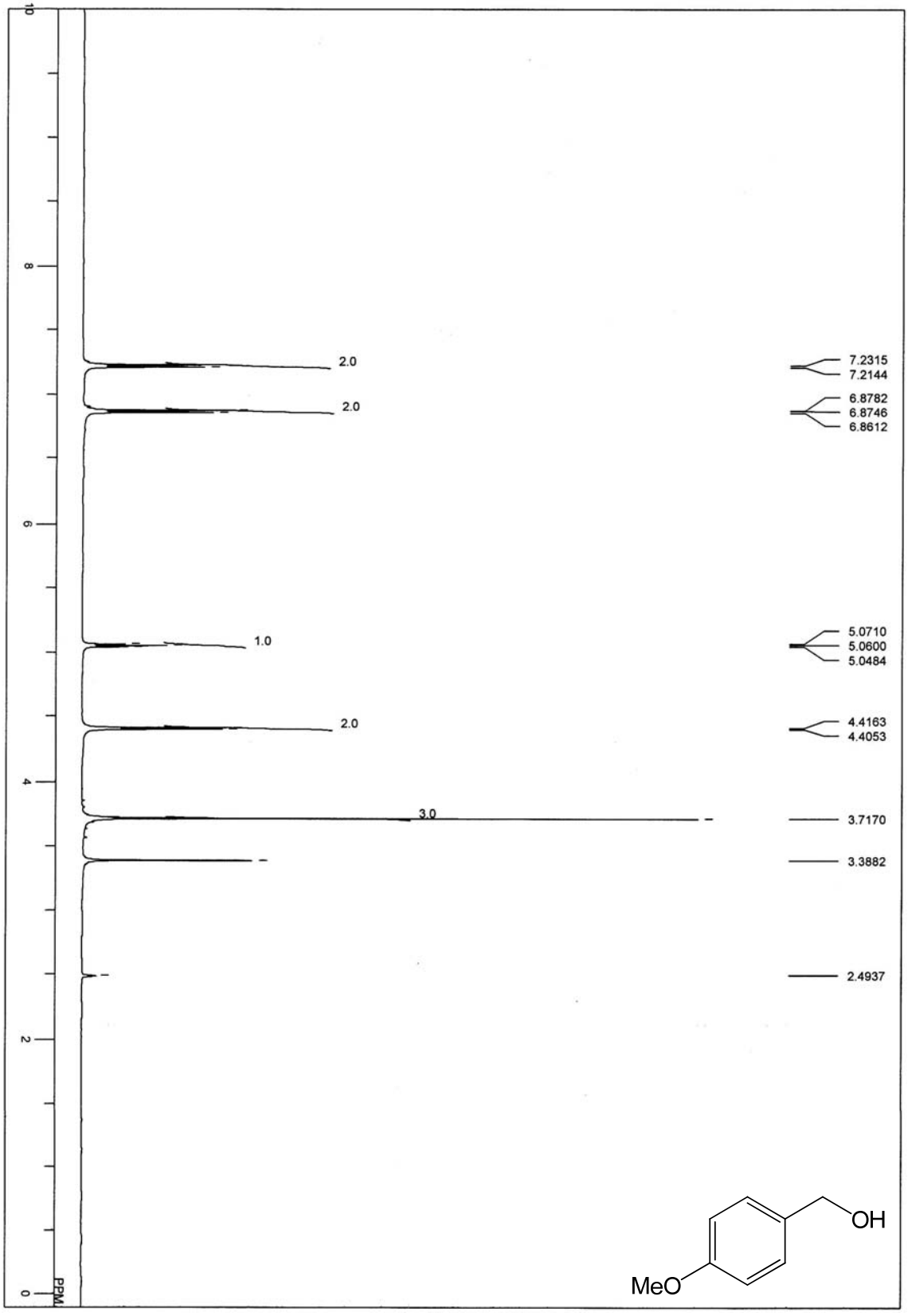
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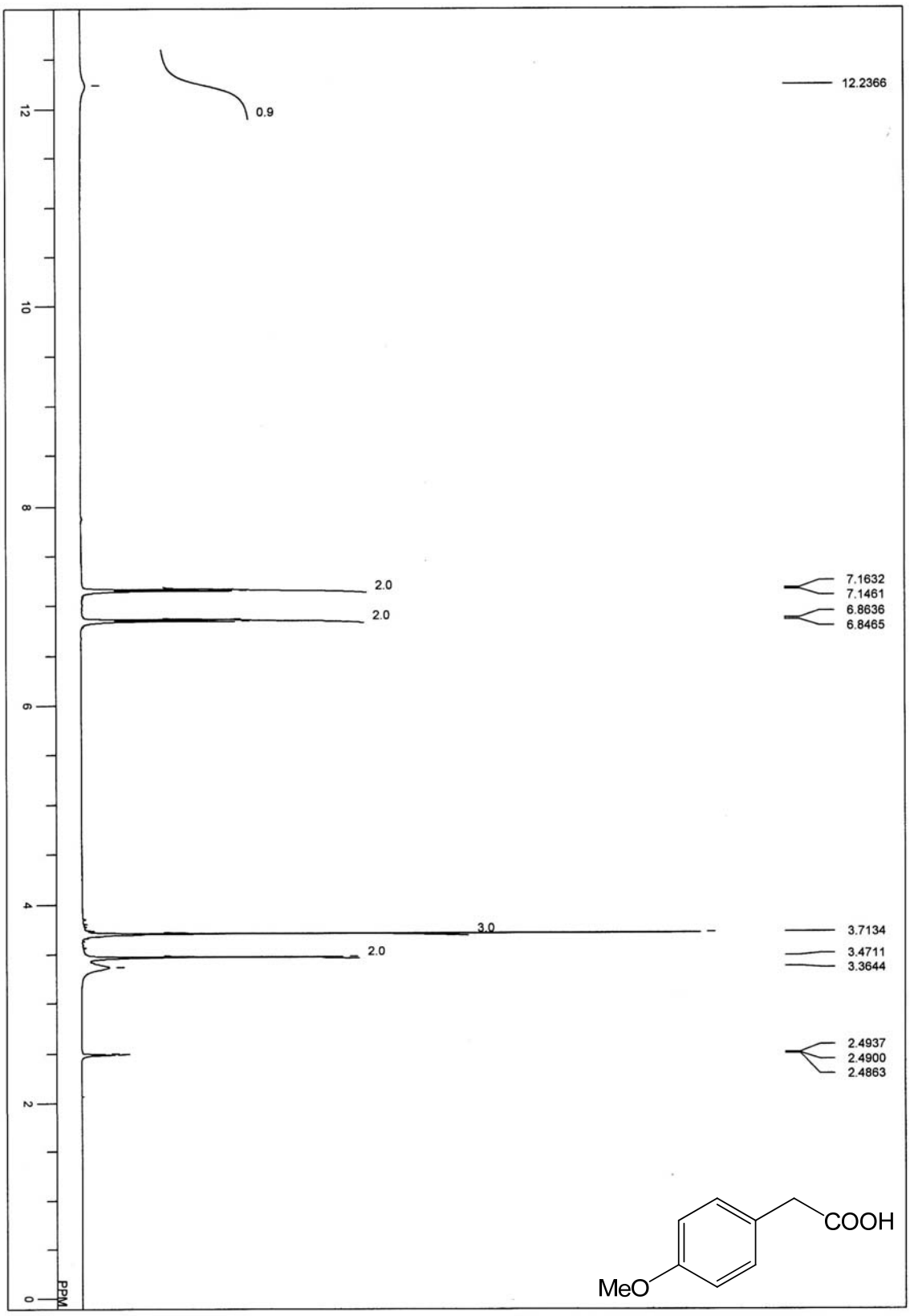
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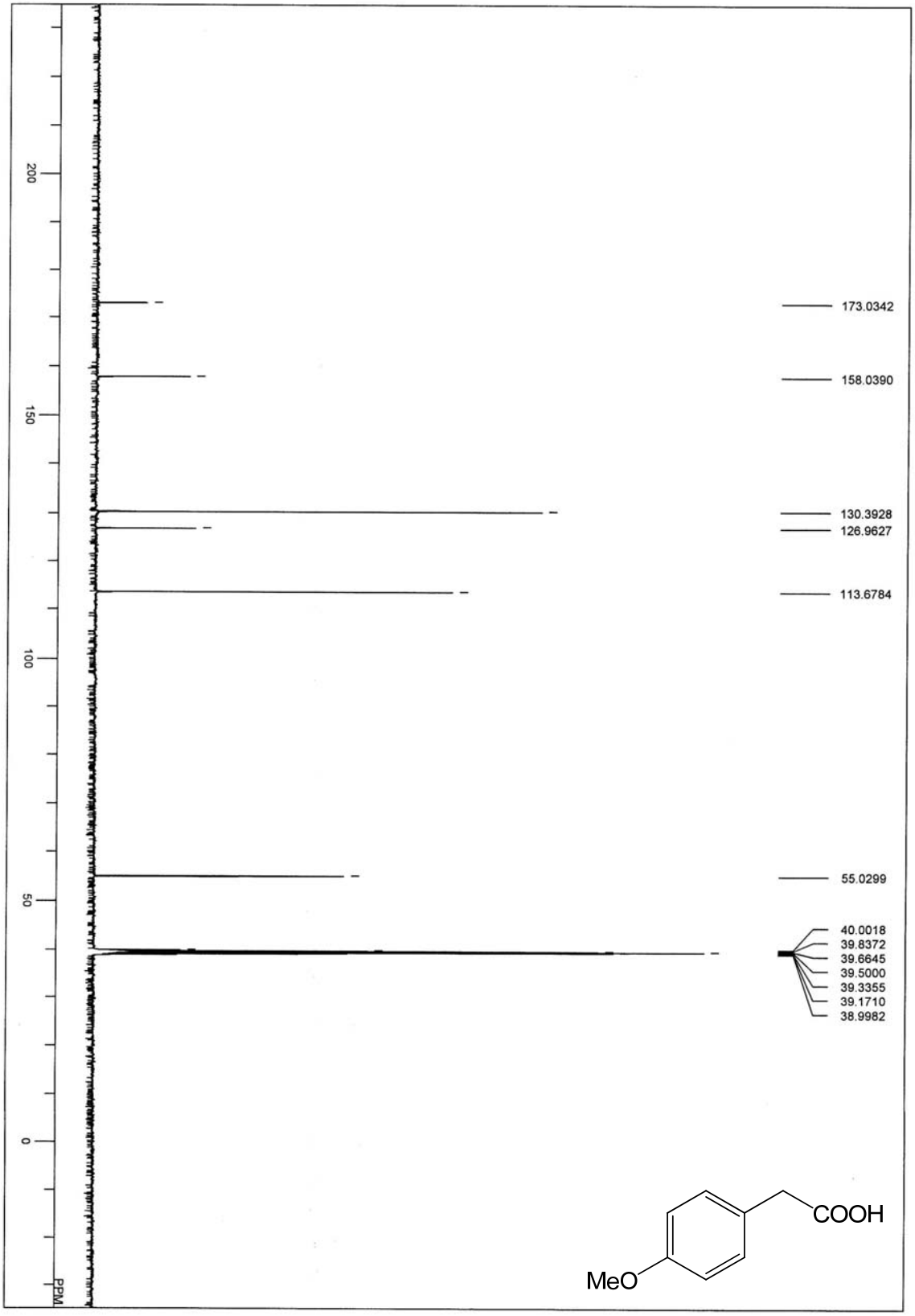
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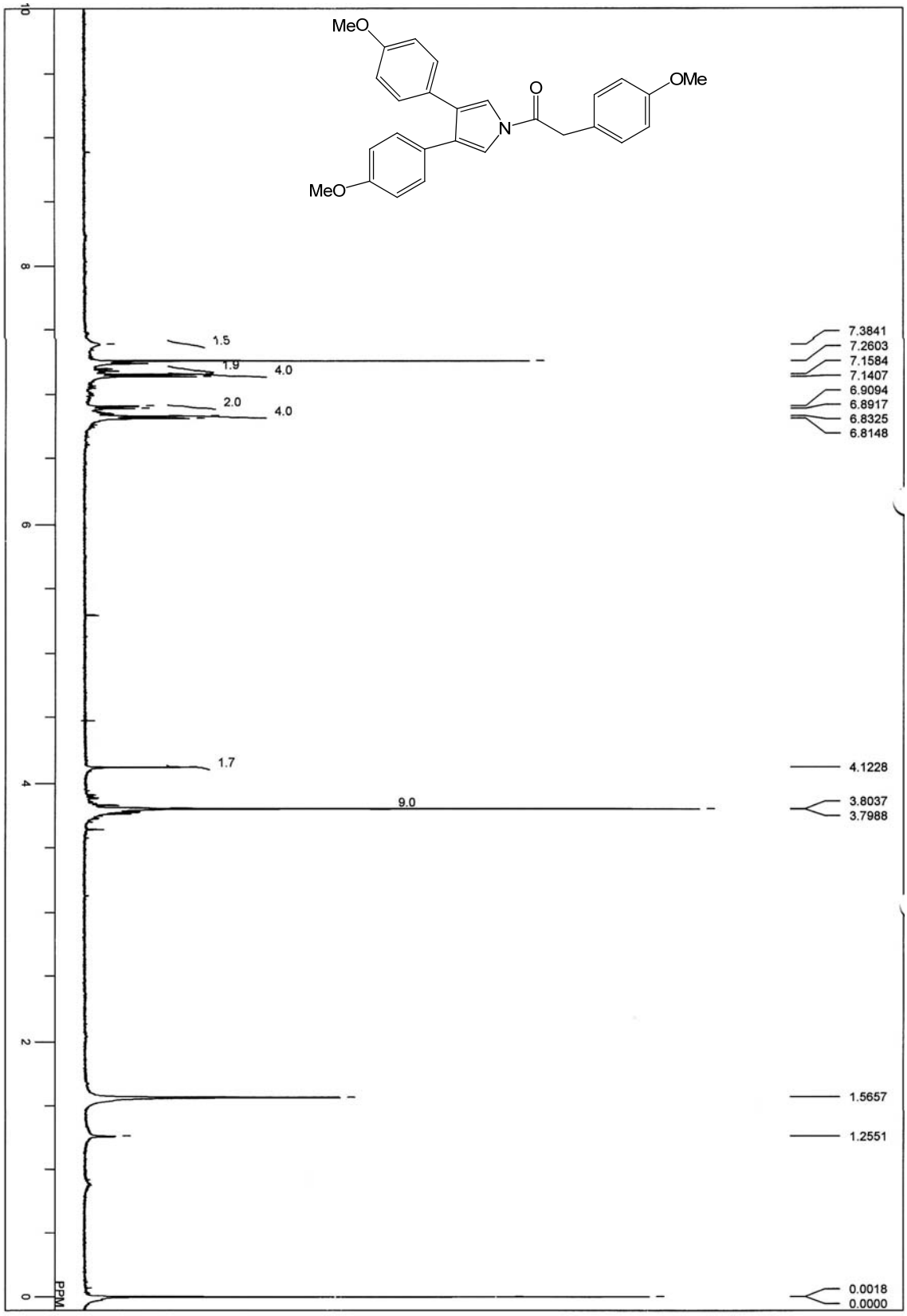
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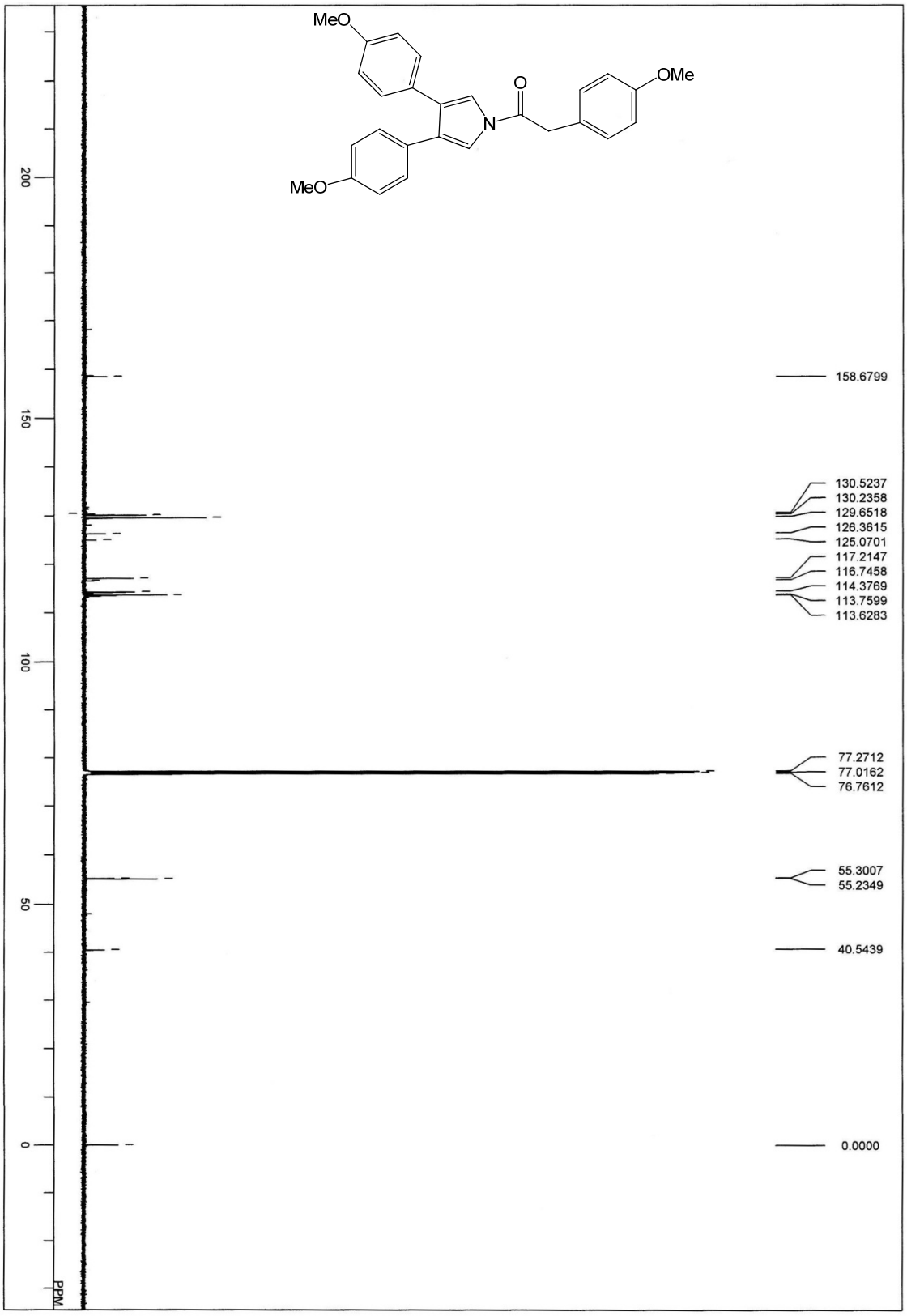
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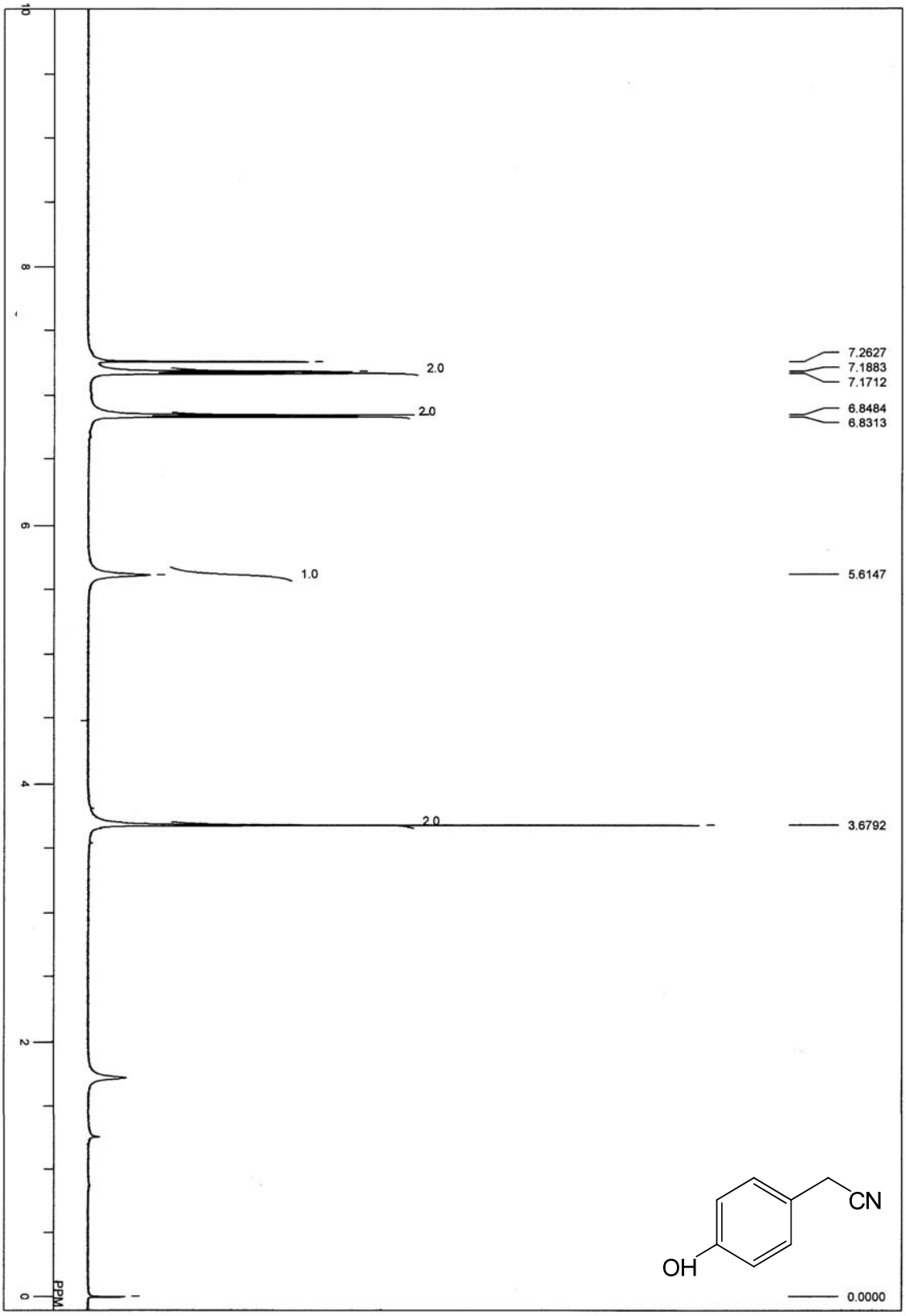
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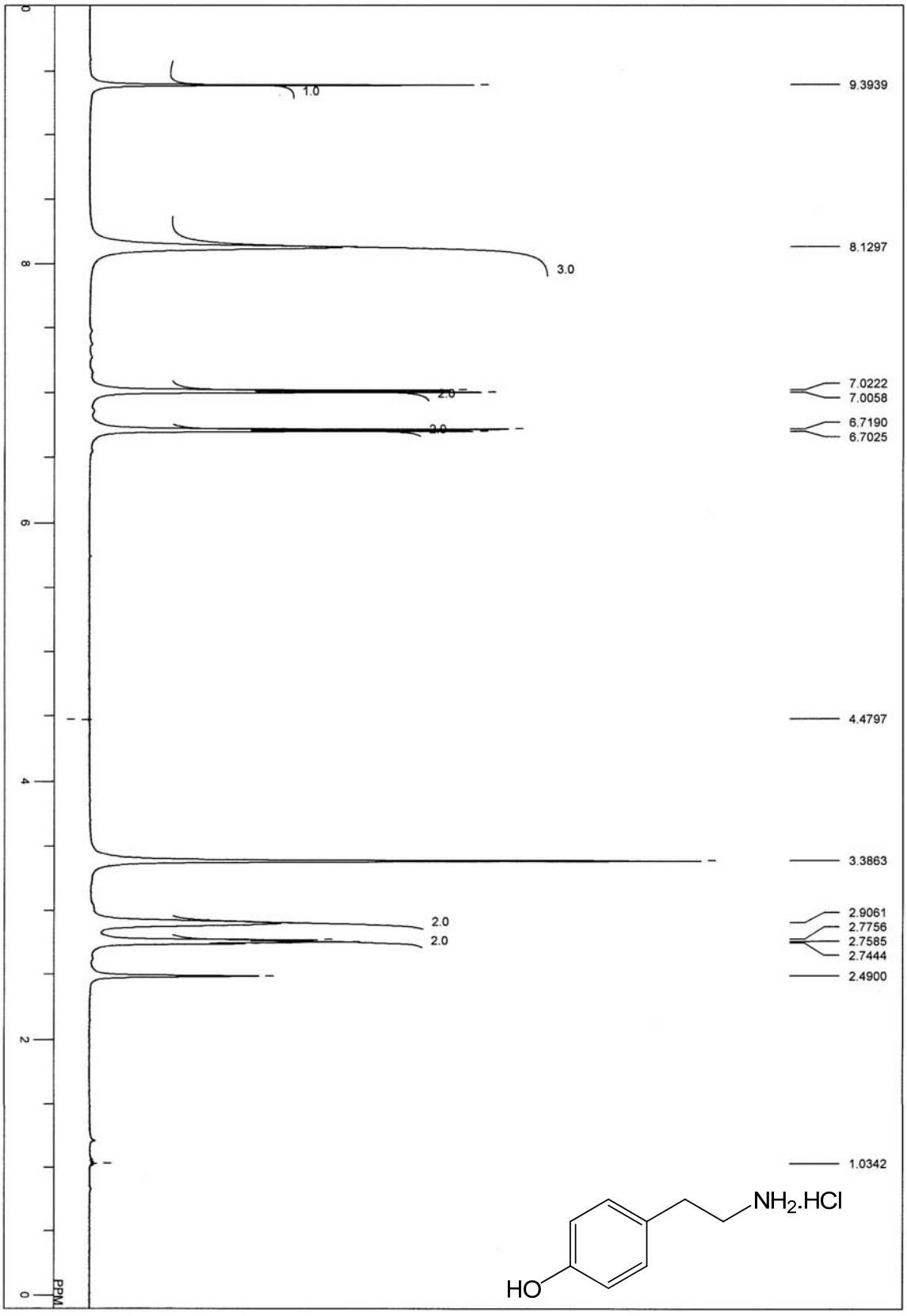
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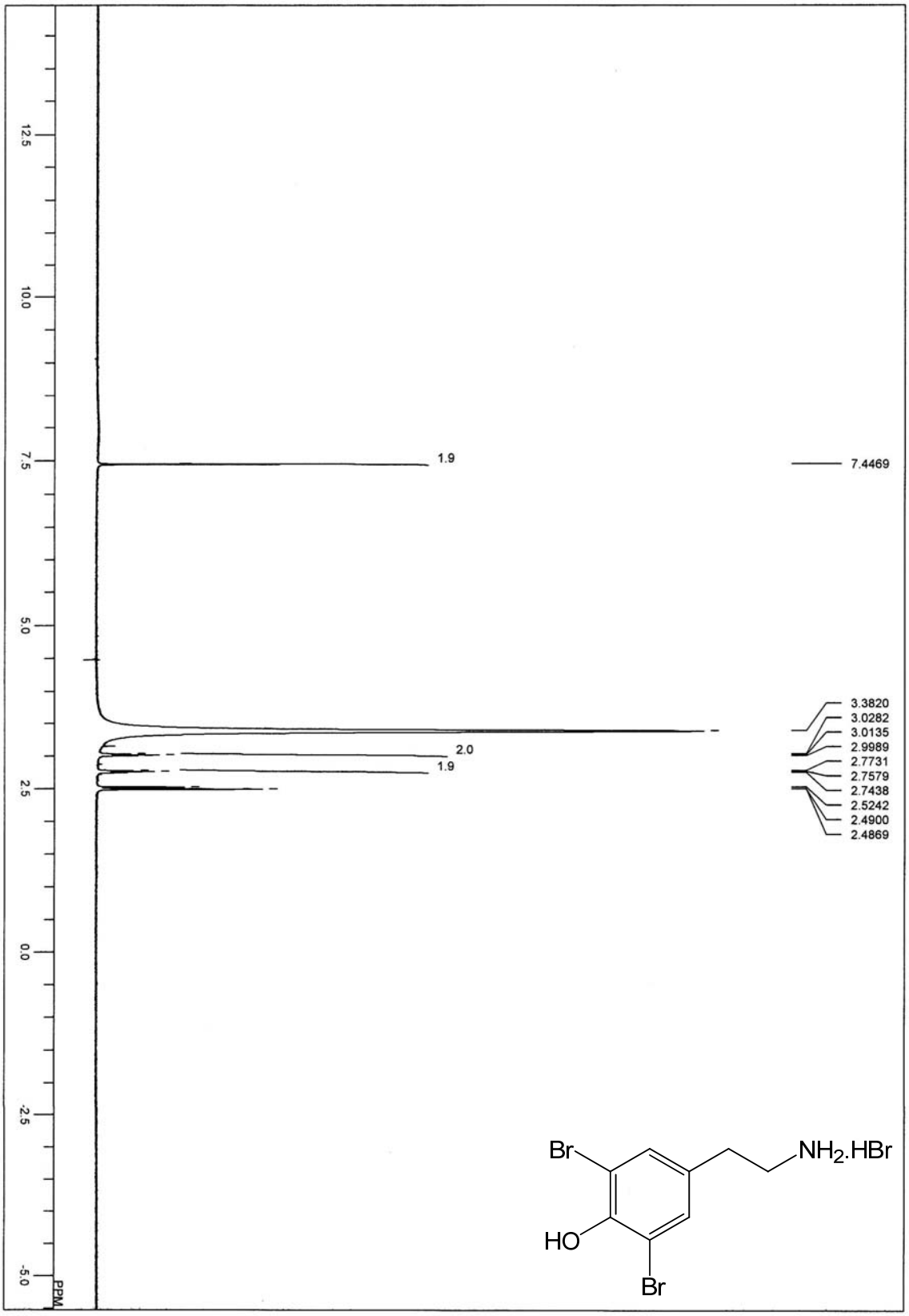
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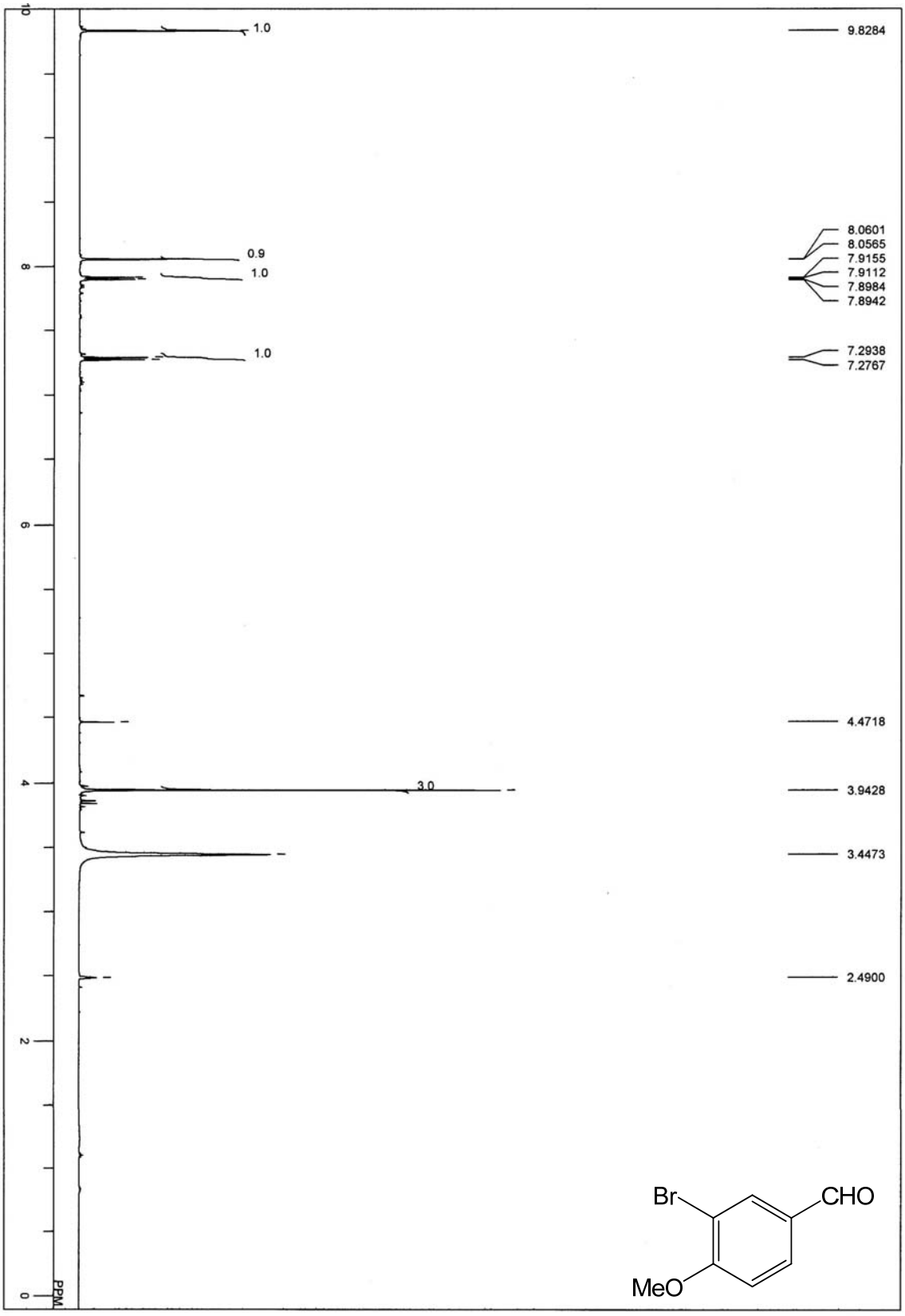
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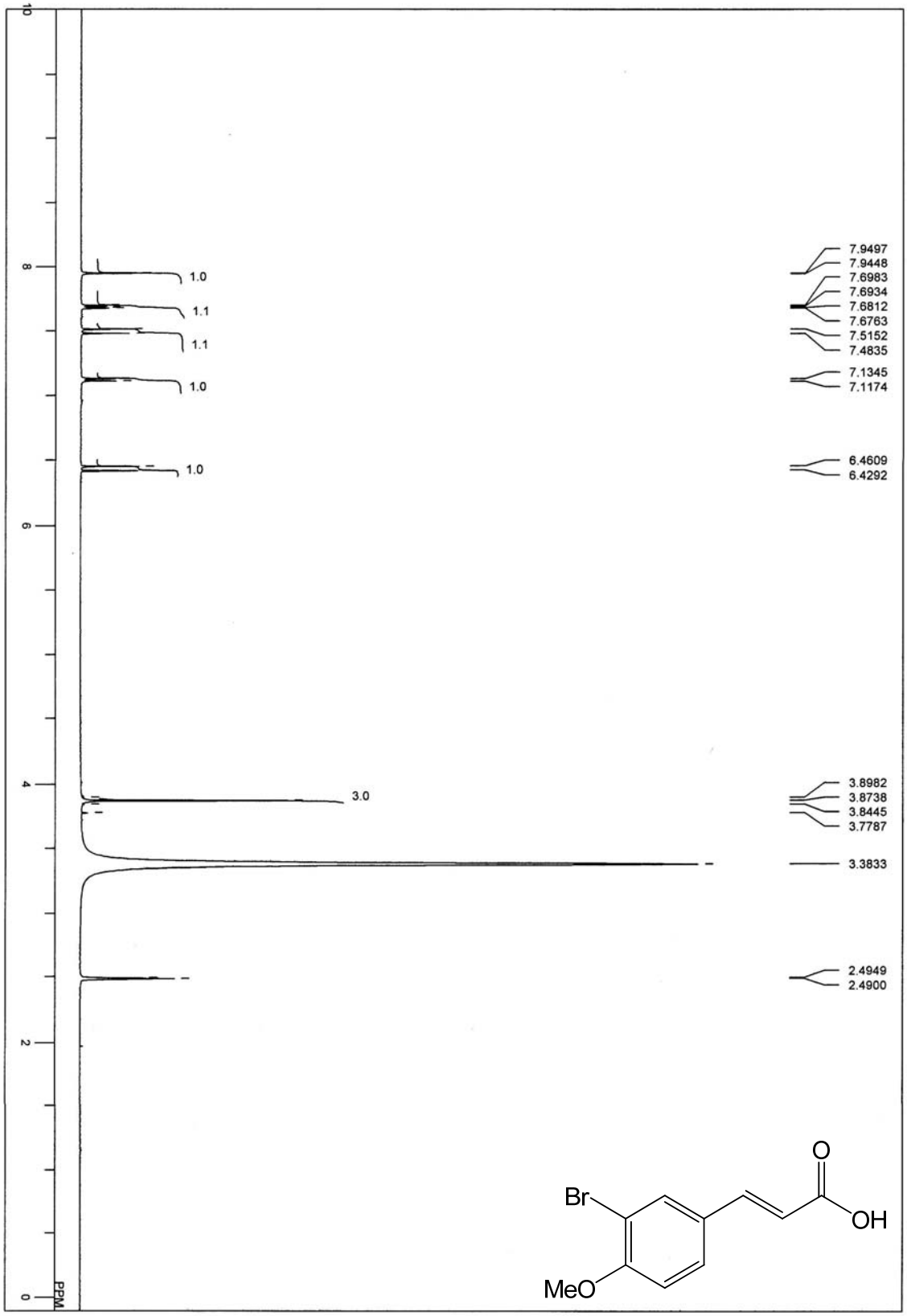
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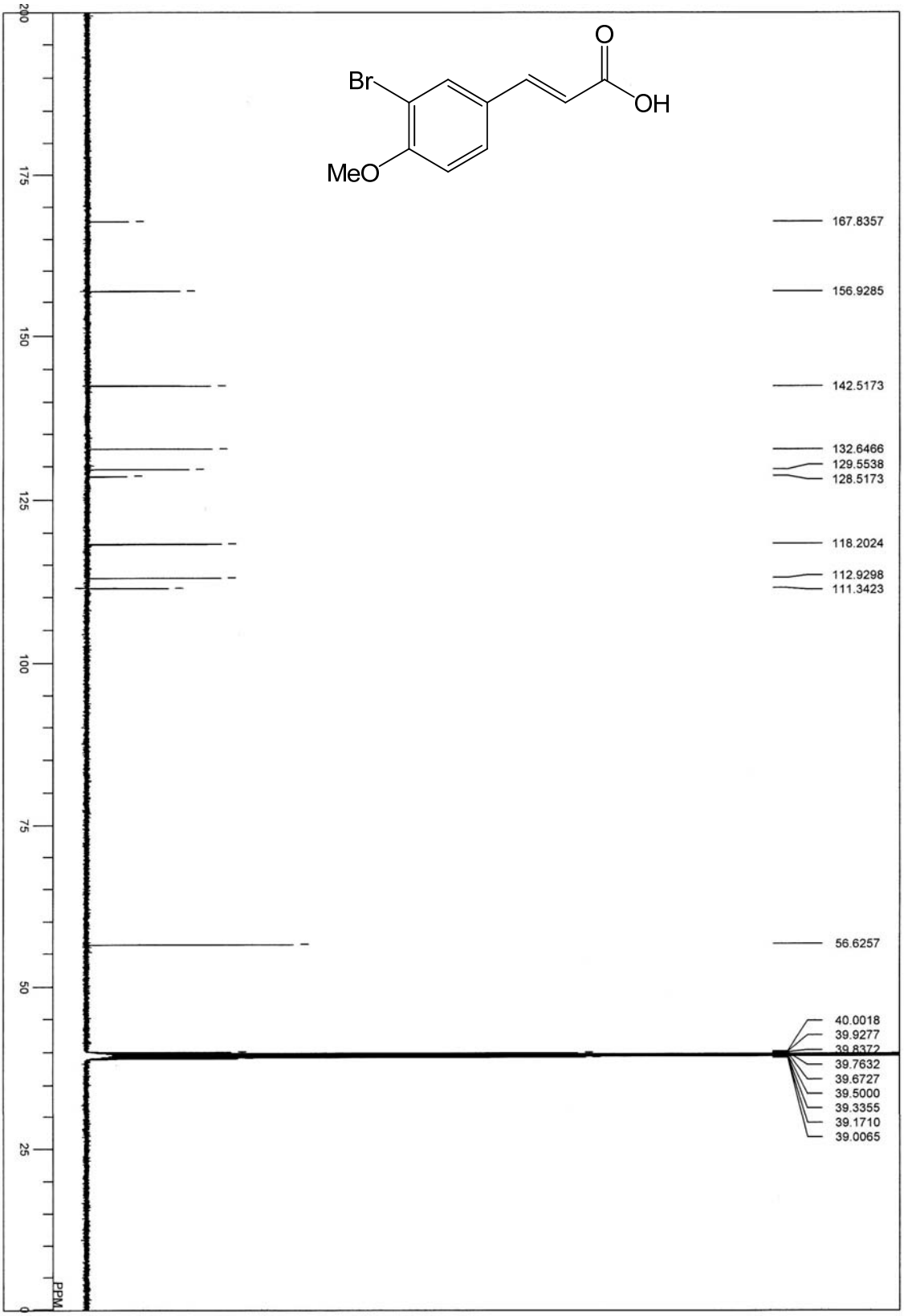
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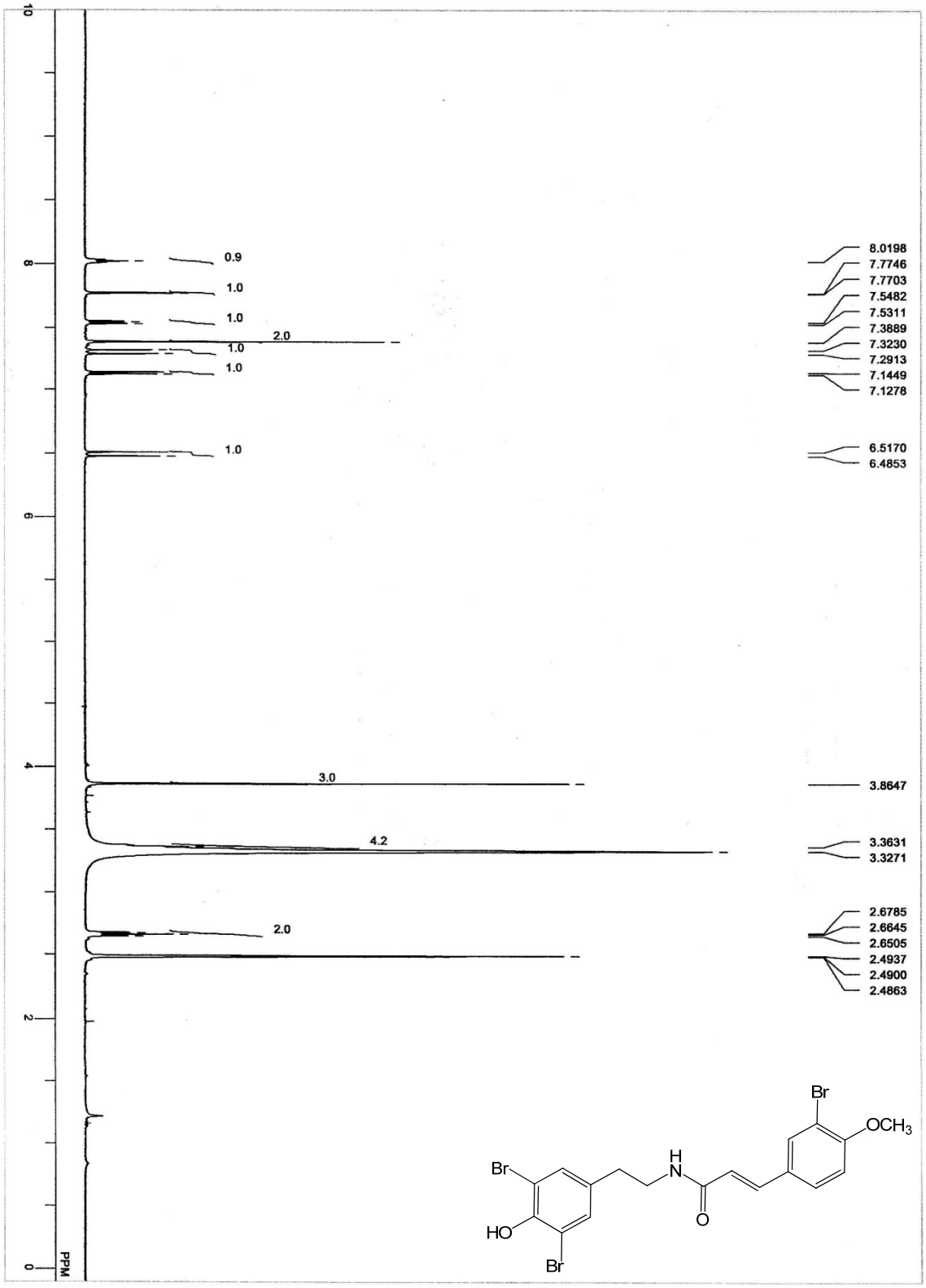
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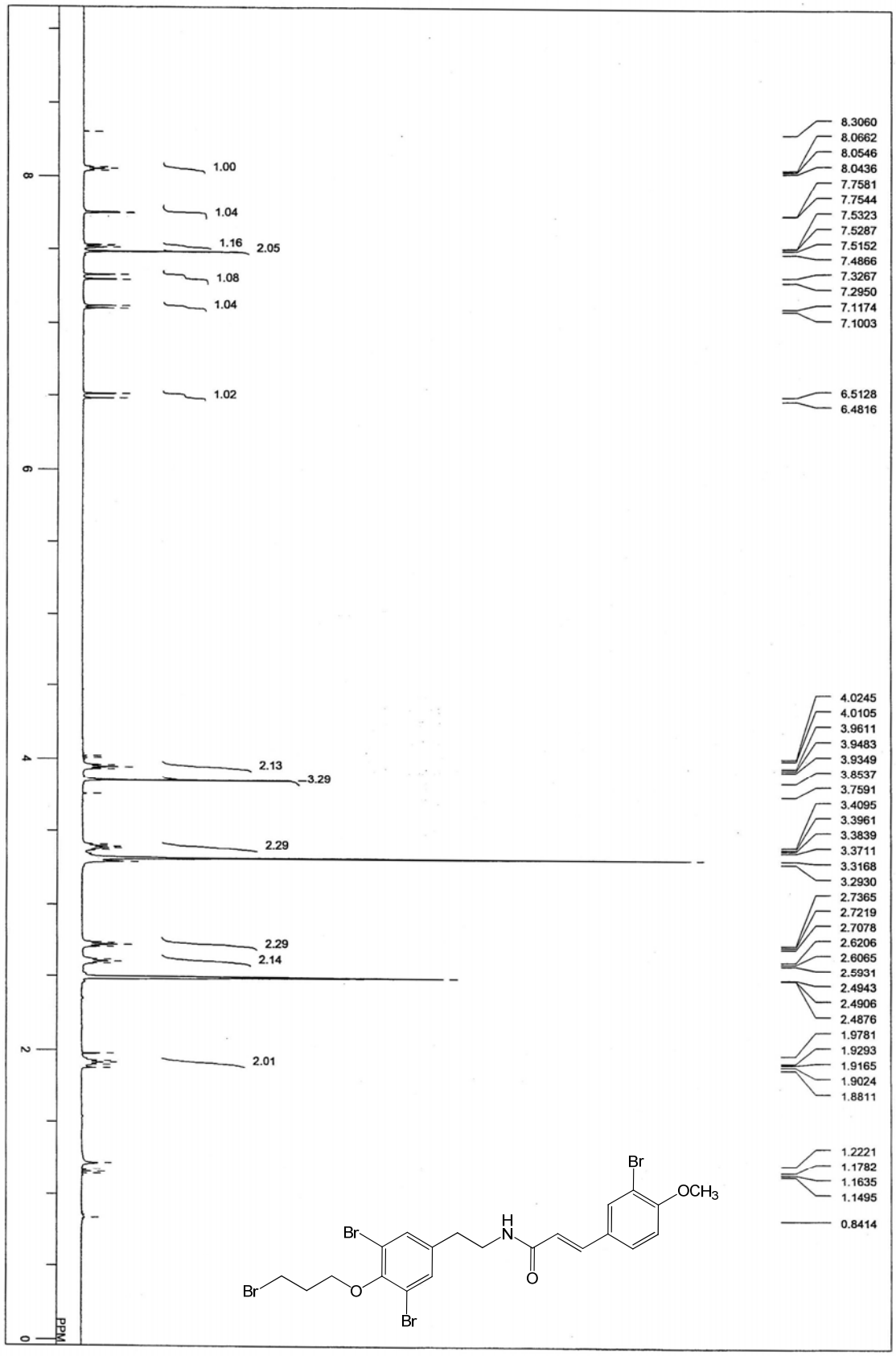
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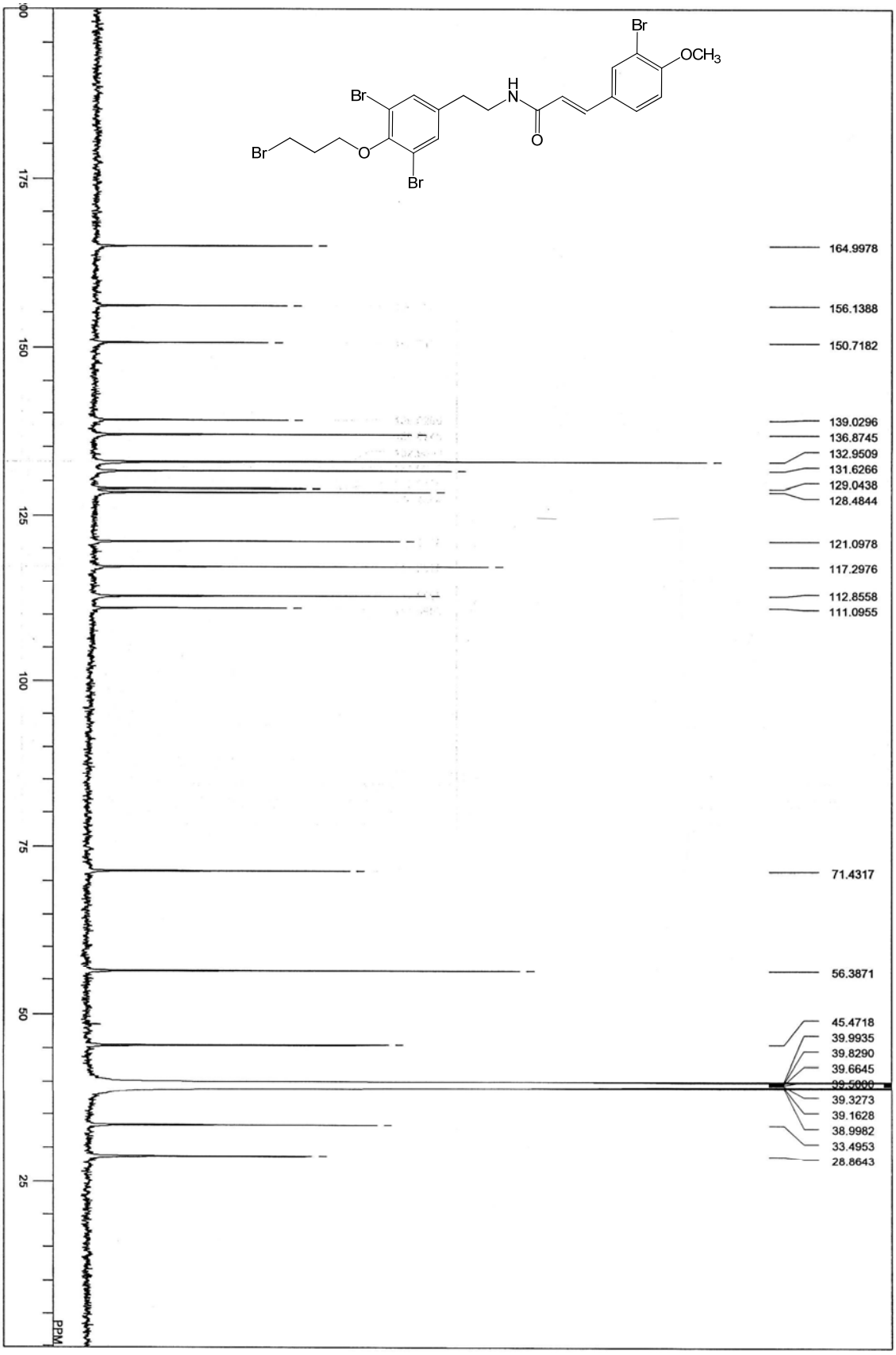
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PPM

# VITA

- M. Khaled Arafeh.

- Born in Damascus, Syria, September 28th 1981.

- Received B.Sc. in Applied Chemistry from Damascus University, Syria, in September 2003.

- Received Diploma certificate in Chemistry from Damascus University, Syria, in September 2005.

- Joined Chemistry department, King Fahd University of Petroleum and Minerals, as Research Assistant in February 2006.

- Awarded M.S degree in Chemistry from KFUPM in March 2009.

- Co-author on the following publication:
  - Nisar Ullah, Khaled M. Arafeh.
    "The first total synthesis of aplysamine 6, an inhibitor of isoprenylcysteine carboxy Methyltransferase".
    Tetrahedron Letters 50 (2009) 158–160.