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Synthesis and Preliminary Biological Screening of Bis-aroyl Methanes and Chemically-modified Curcumins as Matrix Metalloproteinse Inhibitors

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Abstract of the Thesis

Synthesis and Preliminary Biological Screening of Bis-aroyl Methanes and Chemically-modified Curcumins as Matrix Metalloproteinse Inhibitors

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Matrix Metalloproteinases (MMPs) are a family of more than 25 structurally-related enzymes that can degrade a variety of substances including collagen. Basal levels of these enzymes play an important role in tissue turnover under normal physiological conditions, while excessive levels of these enzymes may cause or exacerbate many diseases related to tissue loss, including arthritis, cancer, cardiovascular disease, coma, periodontal disease, stroke etc. For decades, research teams from both industrial companies and academic institutions have been trying to develop an effective matrix metalloproteinase inhibitor as a drug to treat MMP-involved diseases. Our group have been working on MMP inhibitor development for several years and two families of compounds which possess 1,3-dicarbonyl functionality have been investigated. These two families are bis-aroyl methanes (BAMs) and chemically-modified curcumins (CMCs).

Up to now, 23 CMCs have been synthesized and the results published, CMC 2.24 [1,7-bis-(4-hydroxyphenyl)-4-phenylaminocarbonyl-1E,6E-heptadien-3,5-dione] exhibits excellent inhibitory effect against a series of chosen MMPs. Further indications suggested that modification on the 4-substituent of CMC 2.24 could increase both the inhibitory effect and water-solubility. Having this in mind, a 4-hydroxy derivative of CMC 2.24 was synthesized in addition to the four other CMCs. BAMs are the bis-nonvinylene analogues of CMCs, and eight new BAM compounds were synthesized and seven of them were tested in vitro as inhibitors against MMP-9. The synthesis of these five CMCs and eight BAMs as well as the biological screening of seven BAMs will be discussed in this work.

Dedication

This thesis is dedicated to my parents, Mr. Xiaochun Li and Ms. Liqiu Chen, for their long and steadfast love, support and care since my birth till now. Without them, I couldn't complete this work.

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List of Abbreviations

^{1}H-NMR: Proton Nuclear Magnetic Resonance

ATR: Attenuated Total Reflectance

BAM: Bis-aroyl Methane

CAM: Cell Adhesion Molecule

CMC: Chemically-modified Curcumin

CMT: Chemically-modified Tetracycline

DCM: Dichloromethane

DMF: Dimethylformamide

DMSO: Dimethyl Sulfoxide

Dpa: N-3-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl

DPPA: Diphenylphosphoryl Azide

ECM: Extracelluar Matrix

ESI-MS: Electrospray Ionization Mass Spectrometry

EWG: Electron-withdrawing Group

FDA: Food and Drug Administration

GC: Gas Chromatography

IC_{50}: The Half Maximal Inhibitory Concentration

IR: Infrared Spectroscopy

Mca: (7-methoxycoumarin-4-yl)acetyl

MMP: Matrix Metalloproteinase

MP: Melting Point

MT-MMP: Membrane-type Matrix Metalloproteinase

MS: Mass Spectrometry

N/A: Not Applicable

PPTS: Pyridium para-toluenesulfonate

PTSA: Para-toluenesulfonic Acid

RA: Rheumatoid Arthritis

TC: Tetracycline

TEA: Triethyl Amine

THP: Tetrahydro-2H-pyran

TIMP: Tissue Inhibitors of Matrix Metalloproteinase

TLC: Thin-layer Chromatography

TMS: Tetramethylsilane

ZBG: Zinc-binding Group

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For three years I have also worked as a graduate teaching assistant. During these three years, I worked with Professor Frank Fowler, Professor Joseph Lauher, Professor Robert Schneider, Dr. Zachary Katsamanis, Dr. Mohammed Akhtar, Dr. Troy Wolfskill, Dr. Susan Oatis, Dr. Bradford

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Last but not least, I want to say thank you so much to my family members. I love you always!

Chapter 1 General Introduction

Matrix Metalloproteinases (MMPs) are a family of more than 25 structure-related, zinc-dependent proteins which degrade tissue components including collagen and gelatin. The first member of its kind, namely MMP-1, also termed Collagenase-1, was discovered in 1962 by Gross *et al.* It can degrade triple helical collagen fibrils into two fragments, with one approximately triple the size of the other, under physiological temperature and pH [1]. Since then, subsequent discoveries of MMPs have enlarged this family to its current size, and research has shown that this group of proteinases can degrade a variety of constitutes of the extracellular matrix (ECM). The function of MMPs can be either good or harmful, depending on their concentration. When the MMP concentration is low, it can participate and assist normal physiological processes of tissue turnover. However, when MMPs are excessively expressed, the high level of MMPs can overly degrade connective tissue, causing its destruction, and thus leading to or exacerbating a series of diseases including arthritis, cancer, cardiovascular disease, periodontal disease and post-menopausal osteoporosis [2-4]. Therefore, to develop an effective MMP inhibitor has been a goal for both industrial companies and academic labs for decades [2,5].

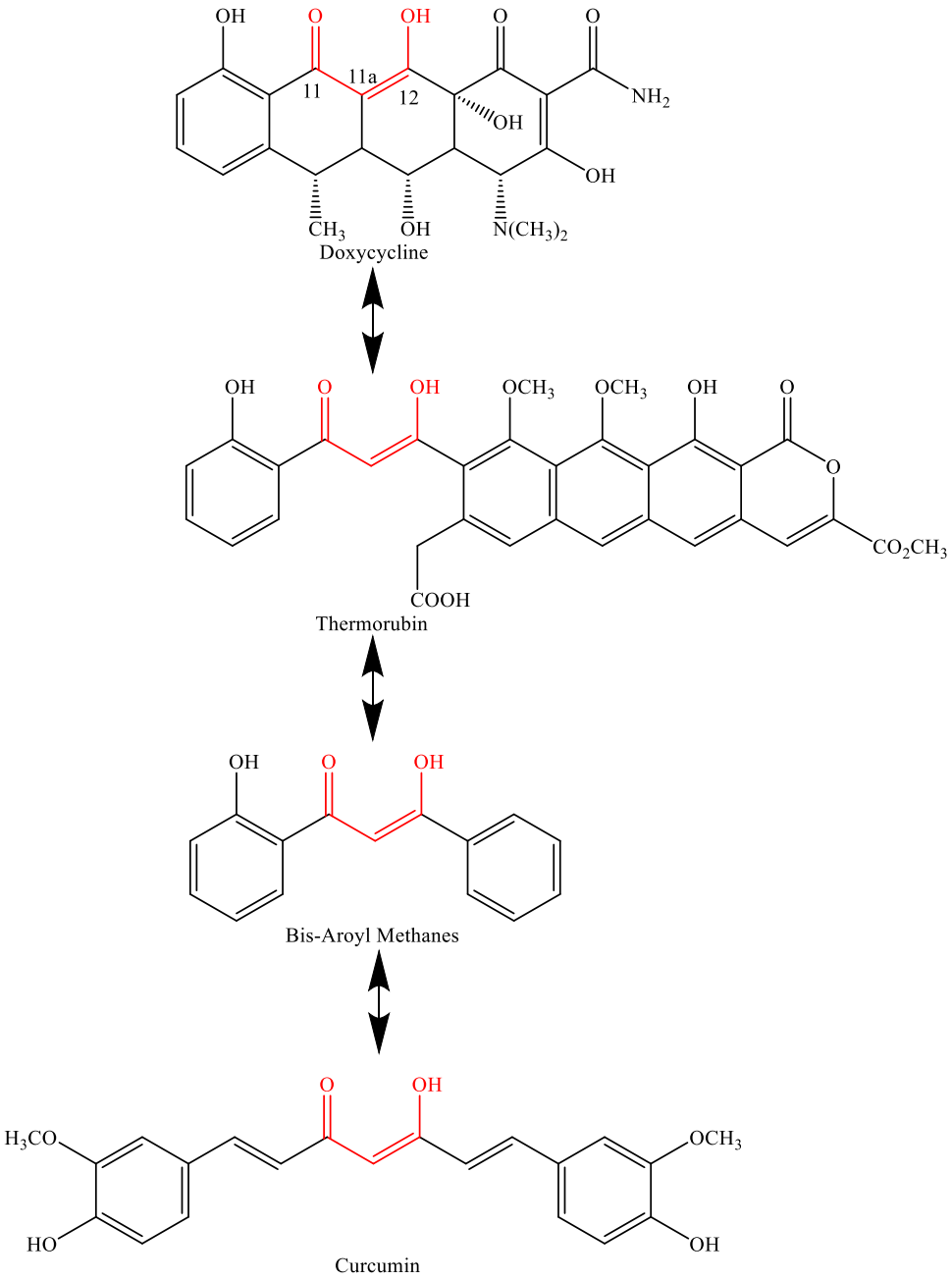
Up to now, the only FDA-approved MMP inhibitor drug available to clinicians for the treatment of periodontitis and chronic skin disease are a number of formulations of sub-antimicrobial doses of doxycycline. The development of this drug occured because of the discovery made by Golub *et al*. in 1983, that an old family of drugs, namely the tetracyclines (TCs) (Figure 1.1), previously used for anti-microbial purposes, were found to inhibit mammalian collagenases and several other MMPs via a non-antimicrobial mechanism [6-9]. With this in mind, besides two FDA-approved orally available drugs, Periostat® for the treatment of periodontitis and Oracea® for

chronic inflammatory skin disease, several chemically-modified tetracyclines (CMTs) were synthesized, without anti-microbial activity but maintaining anti-MMP function. However, CMTs as well as natural TCs, were found to have the intolerable side-effect of increased level of photosensitivity at higher doses, which may prevent it from long-term use [10,11]. Thus only low-dose formulations are used.

Studies had also shown that the enol/β-diketone group of TCs at C-11, C-11a and C-12 positions, is responsible for the binding to the zinc ion of MMPs in the active site, which is the core of MMP catalytic function. So other compounds with similar functionality as TCs may display similar or same inhibitory effects against MMPs. Thermorubin, a natural product isolated from a thermophilic fungus *Thermomycetes* in the countryside near Pavia, Italy, which possesses the same enol/β-diketone group as the TCs do, was tested as an MMP inhibitor (Figure 1.1). However, the anti-microbial activity of thermorubin limits its long-term therapeutic use for the risk of building up the drug-resistance of its targets. Bis-aroyl methanes (BAMs) which share the same enol/β-diketone functionality were therefore chosen for synthesis by the Johnson Group of Stony Brook University. Several BAMs were synthesized (unpublished data) and tested as MMP inhibitors, and the results showed that the inhibitory effect of BAMs are reasonable but not better than the TCs, but no toxicity was observed after 5 hours of incubation with monocytes. To improve the biological activity against MMPs, making the compound more water-soluble is the first part of my research. In the meantime, a bis-vinylene homologue of BAMs, namely chemically-modified curcumins (CMCs) synthesized and tested by Dr. Yu Zhang and had shown significant inhibitory activity against MMPs. Among the published 23 synthesized CMCs [12], one compound CMC 2.24 has the best biological activity. Furthermore, unpublished data showed that a slight chemical modification may increase the biological activity, as well as to increase the

water-solubility. Therefore, to synthesize modified CMC 2.24 derivatives, to achieve a compound displaying better inhibition against MMPs and other pro-inflammatory cytokines and chemokines, with increased water-solubility became the second part of my work.

Figure 1.1 Doxycycline, Thermorubin, bis-aroyl Methanes and Curcumin (Zinc-binding Group in Red)



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Chapter 2 Literature Review of Matrix Metalloproteinases and Their Inhibitors

A. General Introduction of Matrix Metalloproteinases (MMPs)

Matrix Metalloproteinases (MMPs) are zinc-dependent neutral proteolytic enzymes, originally discovered in 1962 by Gross *et al.* [1], when they were studying the metamorphosis of tadpoles. They discovered that one enzyme, which was later termed MMP-1, was responsible for the degradation of collagen at physiological pH and temperature. In their study, they found that MMP-1 cleaved the basi triple-helix collagen unit into two fragments, at approximately one quarter the distance from the C- terminus. The degradation of collagen ultimately leads to the loss of the tale in tadpoles. Since then, more and more structurally related proteinases have been discovered and presently there are 25 different MMPs (23 paralogues have been reported in human) [2], all of which were found to degrade a number of components of the extracellular matrix (ECM). In 1968, the inactive form of MMP-1, or its zymogen, Pro-MMP-1, was purified and isolated from human skin [3].

The activity of the MMPs is highly regulated in a series of stages, including gene expression, storage, secretion, activation and inhibition [2]. Both MMPs and their endogenous inhibitors, which are called tissue inhibitors of MMPs (TIMPs) are active forms of MMPs. TIMPs were discovered in 1975 by Bauer et al. and currently there are four members in this family [4]. MMPs play an important role in tissue remodeling in a variety of physiological and pathological conditions, ranging from arthritis, cancer, cardiovascular disease, periodontal disease, stroke and wound healing [5,6]. Though excessively high level of MMPs may exacerbate a disease, it is also recognized that over-inhibition of MMPs is undesirable and may cause musculoskeletal side-effects such as aches, cramps, pain and weakness [7,8]. Therefore, the development of an

effective MMP inhibitor has been the goal for both industrial and academic labs for the past several decades [7,9-12].

B. Classification of MMPs

MMPs can either be classified on the basis of substrate specificity and molecular structure. According to the references [7,11-13], MMPs can be characterized into five classes: collagenases, gelatinases, stromelysins, membrane-type MMPs, and others that are not classified. These are listed in Table 2.1.

Table 2.1 Classification of Matrix Metalloproteinases

(Modified from References [7,11-13])

Group	Enzyme	MMP Number
Collagenases	Fibroblast Collagenase	MMP-1
	Neutrophil Collagenase	MMP-8
	Collagenase-3	MMP-13
	Collagenase-4, χCol4	MMP-18
Gelatinases	Gelatinase A	MMP-2
	Gelatinase B	MMP-9
Stromelysins	Stromelysin-1	MMP-3
	Stromelysin-2	MMP-10

	Stromelysin-3	MMP-11
	Matrilysin	MMP-7
	Matrilysin-2	MMP-26
Membrane-type MMPs	MT1-MMP	MMP-14
	MT2-MMP	MMP-15
	MT3-MMP	MMP-16
	MT4-MMP	MMP-17
	MT5-MMP	MMP-24
	MT6-MMP	MMP-25
Unclassified MMPs	Macrophage Metalloelastase	MMP-12
	RASI-1	MMP-19
	Enamelysin	MMP-20
	CA-MMP	MMP-23
	Epilysin	MMP-28

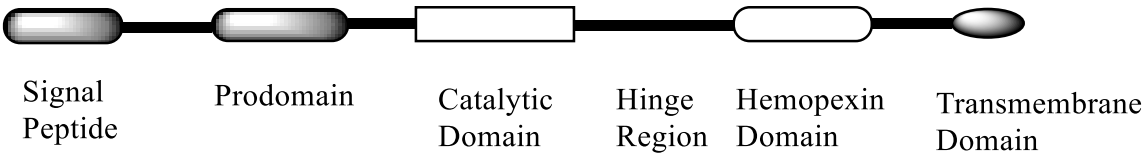
C. Structure of MMPs

All MMPs share some similar structural features but the exact structure varies from one to another. Generally speaking, the structure of MMPs can be segregated into six major structural

domains, from the N-terminus to the C-terminus: the signal peptide (or the signal domain), the propeptide domain (or the prodomain), the catalytic domain, the hinge region, the hemopexin-like domain and the transmembrane domain, illustrated in Figure 2.1. It is reported that MMPs exhibit around 40% identity in their primary structure, and this includes both the propeptide domain and the catalytic domain [12,14].

Figure 2.1 General Structural Domains of MMPs

(Modified from References [12,14])

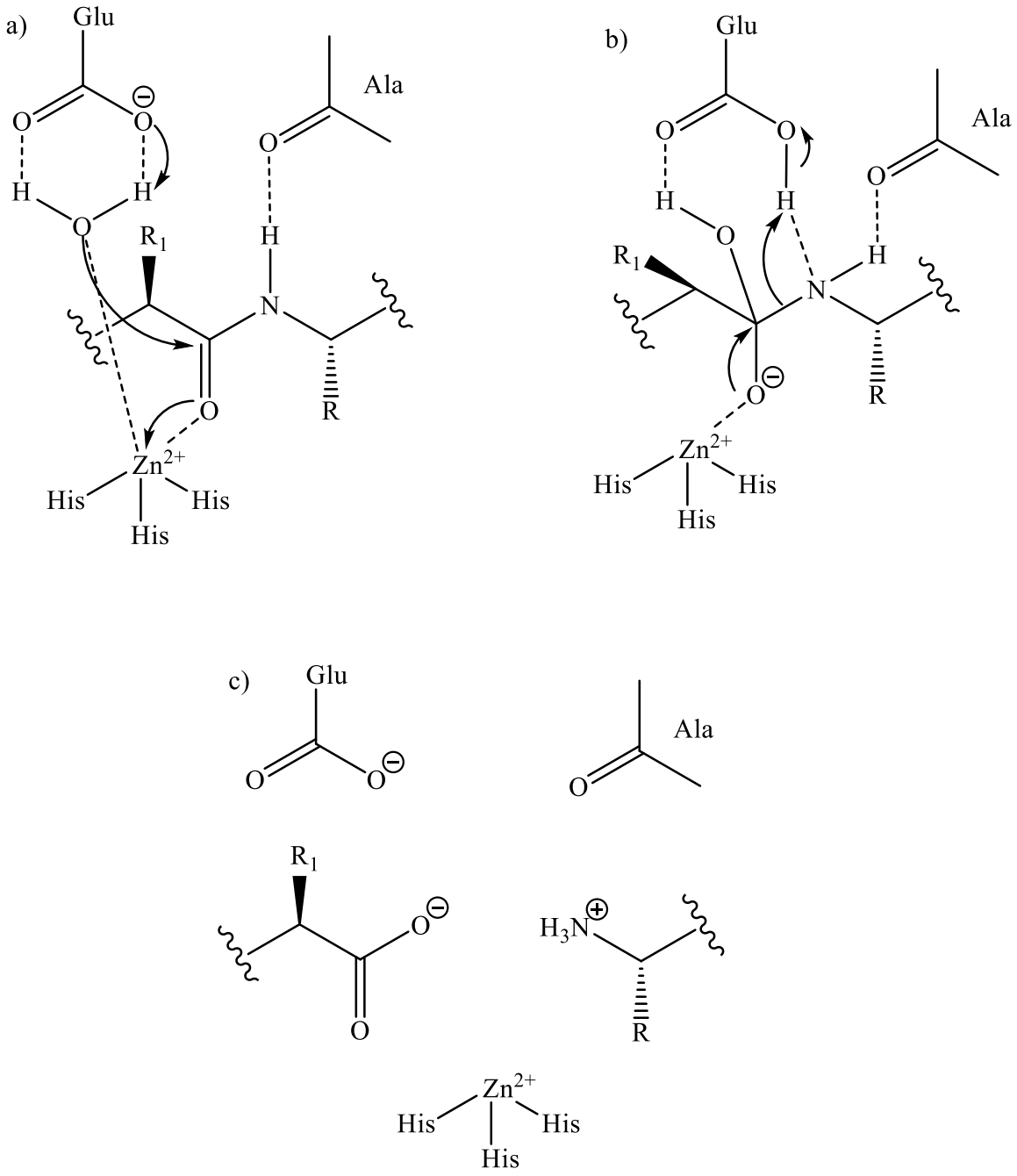


The N-terminus signal peptide is responsible for secretion and for the cleavage of the peptide domain. The prodomain which usually consists of approximately 80 amino acids, is responsible for the enzyme latency [14]. There is a conserved cysteine present in this domain, in the sequence of Pro-Arg-Cys-Gly-Xxx-Pro-Asp. It was found out that this cysteine binds to the zinc ion in the catalytic domain, blocking enzyme hydrolysis of its substrates. Therefore, this domain must be cleaved in order to activate the enzyme [15]. The catalytic region is about 170 amino acids in size, with two zinc ions, one of which is in the active site and the other is associated as a zinc finger (structural). The zinc atom used for catalysis is bound by three adjacent histidine residues, in the sequence motif His-Glu-Xxx-His-Xxx-Xxx-Gly-Xxx-Xxx-His [14]. The catalytic domain is linked to the hemopexin-like domain through the hinge region. This linker likely fulfills important functional roles, not only acting as a physical spacer. For example, the hinge region turns out to be important for the stability of the enzyme and the degradation of substrates.

The hemopexin-like domain is composed of four β–sheets and is frequently involved in substrate recognition/positioning [14]. The transmembrane domain however, is only present in some certain MMPs, and it may serve as a membrane anchor [14].

Once an MMP is fully activated, the enzyme can perform its proteolytic function to cleave a peptide bond. The detailed mechanism is illustrated in Figure 2.2 [16]. In the active form there are three histidine residues that bind the zinc ion, and the forth coordination site is occupied by a water molecule. The water molecule also hydrogen-bonds to a glutamic acid, which activates the water molecule. The carbonyl oxygen of an alanine residue also hydrogen bonds to an amide hydrogen of the peptide (Figure 2.2 a). Once the water attacks the peptide carbonyl in the substrate, one of its hydrogen atoms will be abstracted by the glutamic acid. The newly generated oxyanion will then bind to the zinc. Then the amide nitrogen becomes basic and abstracts a proton from the glutamic acid (Figure 2.2 b). The amide carbon-nitrogen bond then cleaves and the amine will be ionized by the newly formed carboxylic acid, generating a carboxyanion and a primary ammonium ion (Figure 2.2 c).

Figure 2.2 Mechanism of MMP-catalyzed amide bond breaking [16]



C. Regulation of MMP Activities

The activity of MMPs can be regulated at many levels: MMP gene transcription, compartmentalization, or inhibition by protein inhibitors. There is also another regulatory mechanism termed zymogenicity [2]. Before MMPs can perform their physiological effect, they need to be activated. This usually involves a separate enzyme (which may be an active MMP molecule) and which cleaves a fragment from the inactive MMP.

1) MMP Expression

Expression of MMPs in normal cells is usually low, even undetectable [11,17]. Most MMP gene expressions need cytokines and growth factors from external sources [2] in order to operate. MMP genes are expressed when there are physiological requirements, usually peptide cleavage, needed or in some cases where tissue destruction is required. In the latter cases, the expression is usually higher than the homeostatic level. Signaling molecules, for instance, both pro- and anti-inflammatory cytokines, and many other substances including extracellular matrix proteins, bacterial virulence factors and enzymes, and also other factors such as cell stress, cell communication etc. can up- or down-regulate the MMP expression. Based on the gene expression, MMP can be segregated into two categories: constitutive or homeostatic MMPs, the level of which remains the same under all physiological conditions, for instance, MMP-1; and inducible or inductive MMPs, whose level can increase dramatically under certain physiological or pathological conditions, like MMP-9 [6].

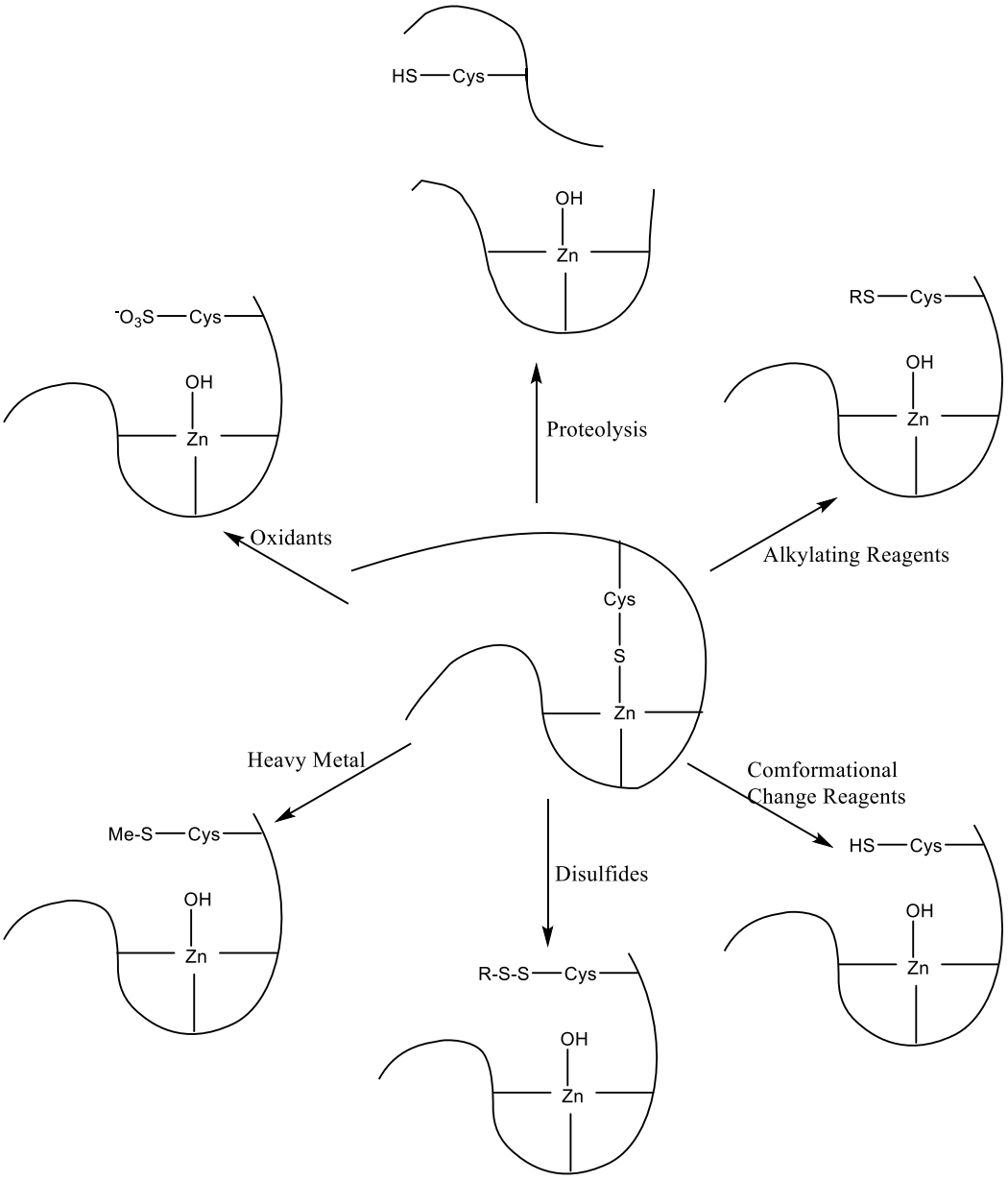
2) MMP Activation

Once MMPs are synthesized, they are stored in inflammatory cell granules. The storage suppresses their availability, normally MMPs are secreted from cells as inactive forms, namely pro-MMPs [2]. Pro-MMPs contain the pro-domain (zymogenic domain), which is between 66

and 91 residues long in those MMPs that have been structurally characterized (Figure 2.2), and will be cleaved during the activating process [18]. The activation can be completed in two steps. Firstly, the pro-domain will be cleaved but one thiol group of a cysteine in the pro-domain backbone will still interact with the zinc ion, which is in the active site. The second step involves complete cleavage of the pro-domain and the Zn-thiol interaction will be terminated. This provides physical space for the zinc ion to coordinate other substances, such as water, to exhibit its catalytic function. This activating mechanism is called the “cysteine switch” [18].

Several methods have been reported so far to break the zinc-cysteine coordination bond, namely: (a) treating it with ions such as mercury(II) or silver(I); (b) treatment with conformation changing agents; (c) cleavage of the zinc-cysteine bond with proteolysis agents, (d) alkylating the thiol group; (e) conversion of the thiol group to a disulfide; or (f) usage of oxidants (Figure 2.3) [18]. The cleavage of the zomogenic domain will convert MMP to its active form.

Figure 2.3 Activation of MMPs by cysteine switch mechanism [18].



3) Endogenous MMP Inhibition

MMP activity can also be regulated by its inhibitors. Tissue inhibitors of matrix metalloproteinases (TIMPs) are the major endogenous inhibitors in tissue that regulate MMP activity. There are 4 types of TIMPs, named TIMP-1 to TIMP-4 [6]. TIMPs are a family of secreted glycoproteins [6], consisting of 184-194 residues and the molecular weight varies from

21 to 30 kDa. TIMPs have 2 domains, the N-terminal domain and the C-terminal domain which are connected by 3 disulfide bonds, with 3 cysteine residues being on one each domain [14]. The specificity and the inhibitory effect of these four TIMPs vary. For TIMPs 1-3, TIMP-1 is more specific for MMP-9, TIMP-2 is more specific for MMP-2 and TIMP-3 exhibits almost equal inhibitory effect against both MMP-2 and MMP-9 [19]. Of these the 4 TIMPs, TIMP-3 can modulate the activity of MMP more effectively than the other TIMPs due to its direct linkage to the extracellular matrix (ECM) components (this feature distinguishes TIMP-3 from the other 3 TIMPs), although no TIMP-1 or TIMP-3 is found in the interstitial space [14]. TIMP-4 is detected in high concentration in heart tissue but in low concentration in the kidney [14]. TIMPs can bind to MMPs in a 1:1 ratio, by forming a noncovalent complex [14]. This 1:1 stoichiometry suggests that there is a balance between TIMPs and MMPs, disruption of which may lead to a variety of pathogenic processes.

D. MMPs and Diseases

It was mentioned earlier that the level of MMPs is highly related to diseases. While the basal level of MMPs are necessary for normal physiological function such as tissue turnover [6,12], elevated level of MMPs may overly degrade tissue components and thus cause or exacerbate other diseases [5,8,11,12,20,21]. It is found that the excessively high level of MMPs are linked with different diseases including but not limited to arthritis, cancer, cardiovascular disease, inflammatory disease, neural plasticity disease and postmenopausal osteoporosis, all involve tissue destruction. In this thesis, I will briefly discuss the roles MMPs play in arthritis, cancer, neural plasticity disruption and inflammatory disease.

1) MMPs in Arthritis

One MMP in the MMP family, MMP-3 had been found to be the major tissue destroyer in rheumatoid arthritis (RA) due to its upregulation in contrast to other MMPs, and it is mainly produced by synoviocytes [22]. Studies have also suggested that excessive levels of MMP-3 in the serum and synovium correlate well with the RA of patients, as well as being a predictor of the degree of joint destruction in RA [23-31]. Several other Pro-MMPs such as Pro-MMP-1, Pro-MMP-8, Pro-MMP-9, etc can also be activated by MMP-3 [32]. These results mentioned above support the notion that MMP-3 levels might be a promising candidate as a new biological marker for RA diagnosis [33].

2) MMP in Cancer

The impact of MMP on cancer was first notices in the early nineties [34,35]. To date, much research data supports the idea that MMPs are involved in many steps of tumor pathology, such as: tumor growth, tumor protection, apoptosis, angiogenesis, and metastasis.

MMPs have been found to be necessary to tumor cell growth. MMPs can both mediate the release of cell growth factors [34] and activate those factors that are normally anchored to the cell surface or attached to the peritumor ECM. Some clinical studies have shown that MMPs 1, 2, 7, 9, 11, 13 and 14 are more highly expressed in tumor cells than in normal cells. It is also clear that MMPs have a direct effect on cell growth [36].

It is of vital importance for tumor cells to protect themselves against the mammalian immune system. MMPs have been found to be involved in a series of escape mechanisms that are developed by tumor cells [9,37,38]. Besides these mechanisms, some MMPs can also protect the tumor cell by other methods. For example: MMP-9 can disrupt and eventually suppress the rapid

growth of T-lymphocytes [39] whereas MMP-11 makes the tumor cells less sensitive to human natural killer cells [40]. Moreover, MMPs can also stimulate the protective and adaptive responses of their host [41].

The apoptotic effect caused by MMPs in tumor pathology has also gained considerable attention. MMPs have a broad effect on the apoptosis of both normal cells and cancer cells. On the one hand, MMP-3 and MMP-7 have been found to induce pro-apoptotic or apoptotic effects on epithelial cells [42], whereas MMP-1, MMP-9, and MT1-MMP can lead to the apoptosis of autophagic cells and also breast cancer cells [43]. Moreover, some substances, such as the Fas ligand, which is bound to the membrane, can be cleaved and released by MMP-7, thus making tumor cell more resistant to chemotherapeutic agent [44].

Tumor angiogenesis, or new blood vessel formation from pre-existing vasculature, is a key step in tumor pathology, and it is also associated with tumor metastasis, which then often leads to the death of the cancer patient. Tumor angiogenesis is completed by using its own angiogenic phenotype, which can be achieved via a process called the angiogenic switch [45]. This is regulated by the balance between pro- and anti-angiogenic factors, which are stored in the ECMs [45]. Therefore, break-down of the ECMs can result in the access to the pro-angiogenic factors, disruption of the angiogenic switch and initiation of angiogenesis. Due to the proteolytic property of MMPs, they can degrade ECMs to release angiogenic factors. The high levels of MMPs in pathological tissues can also cause fibrous protein degradation including collagen and elastin, which are the major structural components of connective tissue and blood vessel walls. This will ultimately lead to the proliferation and migration of endothelial cells and the development of tumor blood-supply capillaries. However, several studies have shown that some

MMPs can also generate angiostatin that inhibits the proliferation of endothelial cells [46]. These results increase the complexity of the roles of the MMPs in tumor angiogenesis.

The action of MMPs in the metastatic phase, is to destroy the tissue around the malignant neoplasm to create space for the proliferating tumor cells and their migration [47]. For example: MMP-9 has been found to be the main factor in the metastatic phase of lung cancer [48], and MMP-2 and MMP-14 are involved in *in vitro* cell migration [49].

3) MMP in Neural Plasticity Disruption

The neurons in the central nerve system play an essential role in controlling human physiological processes as well as learning. The synaptic connections between neurons (i. e., neural plasticity) can be remodeled by factors including MMPs, ECMs and cell adhesion molecules (CAMs). It is found that the ECM-neuron interaction is critical to the synaptic structure, and the remodeling of it requires CAMs [13]. The remodeling of the synaptic structure can interfere with the neural plasticity which can mediate learning and memory [13]. Thus, the degradation of CAMs may affect human memory and learning. The extracelluar domain of a CAM makes it a target for MMP. Therefore, MMPs must be strictly regulated for the protection of CAMs. Several studies have shown that MMP-9 is involved in long-term potentiation, habituation and associative learning [13].

4) MMPs in Inflammatory Disease

MMPs are found to be involved in many inflammatory diseases including asthma, lung fibrosis, hepatitis and periodontal disease. During an inflammatory response, leukocytes need to travel through tissue barriers, and thus, they must be equipped with enzymes that can remodel the ECM

[50], and the MMPs can be used to accomplish this task. As an example their physiological role in periodontal disease will be discussed.

Periodontal disease is a typical inflammatory disease. Currently, it is considered that bacterial dental plaque is the major risk factor that initiates periodontal disease [6]. When bacteria invade the dental plaque, their metabolites initiate the host-cell immune response, producing and releasing proinflammatory mediators [51-53], which starts the destruction of surrounding tissues. Leukocytes and monocyte/macrophages then begin to recruit and amplify the inflammatory response, resulting in the second stage of periodontal disease. The proinflammatory stimuli induce cells in the periodontium and resident periodontal ligament to secret MMPs [52,53]. However, several recent studies have shown that the proteolytic activity of MMPs is not just restricted to the degradation of ECMs, but also affects other non-matrix substrates [6]. This suggests that MMPs may be involved in both tissue destruction and the anti-inflammatory process [6].

E. Matrix Metalloproteinase Inhibitors

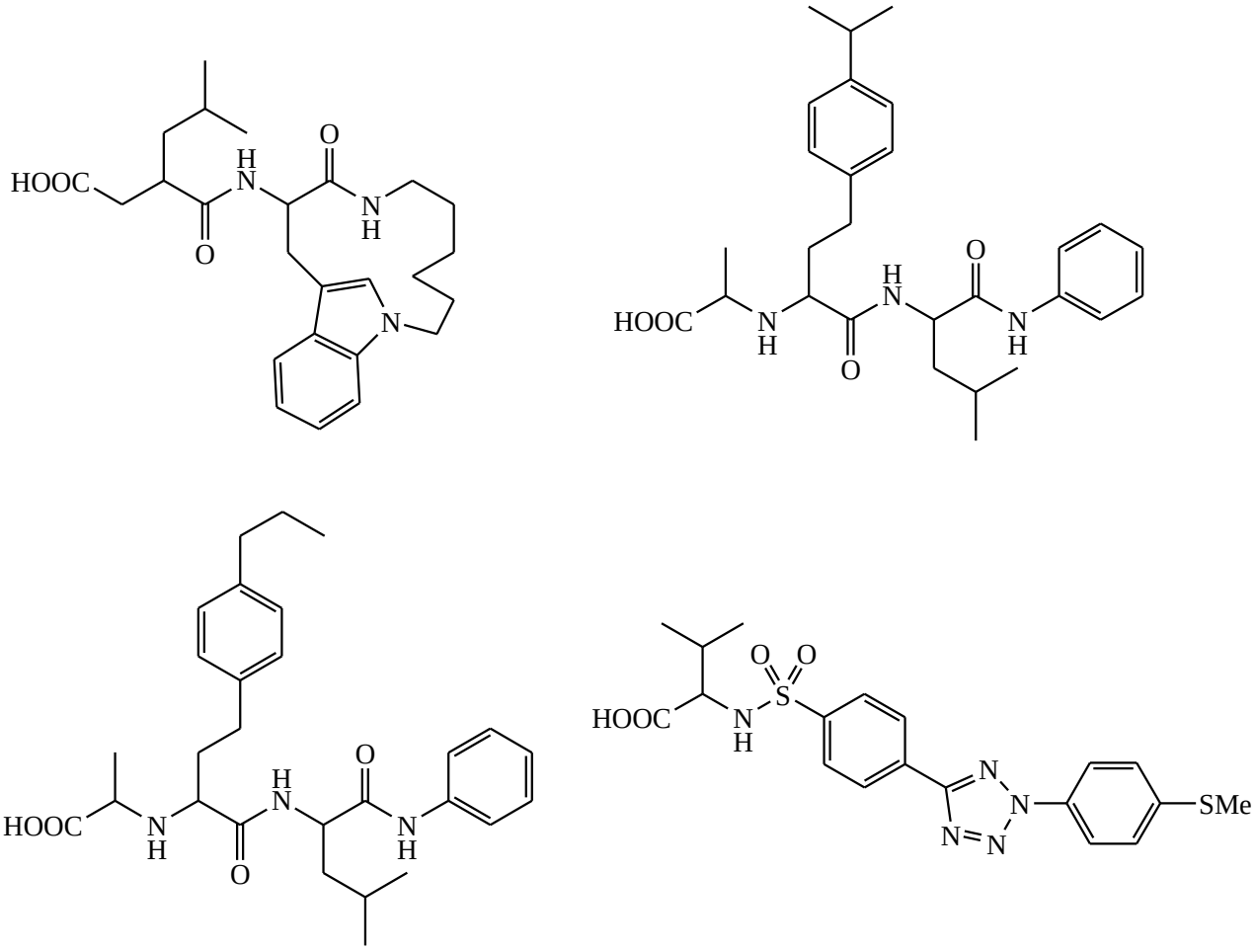
Due to the broad involvement of MMPs in various physiological processes and diseases, many studies have focused on the development of MMP inhibitors as a potential drug for chemotherapy. The vital role of the zinc ion in MMP provides an ideal target for the design of MMP inhibitors. Based on this, the structure of the MMP inhibitors can be categorized into two motifs: the zinc-binding group (ZBG), and the backbone, which has a great influence on the water-solubility, lipid-solubility, binding affinity to the backbone amino acid residues of MMPs. Since in the Pro-MMPs, zinc ion is bonded to a deprotonated cysteine residue, so the synthesized ZBG should also possess some degree of acidity in order to perform the inhibitory function. Up to now, there are several classes of MMP inhibitors published, they are carboxylic-acid-based

inhibitors, thiol-based inhibitors, phosphorus-based inhibitors, hydroxamate class, chemically-modified tetracyclines (CMTs), and chemically-modified curcumins (CMCs).

1) Carboxylic-acid-based Inhibitors

The carboxylic-acid-based inhibitors use COOH as the ZBG. Although they are among the weakest MMP inhibitors, they are less toxic than other synthesized inhibitors and have good oral bioactivity. They usually contain bulky, hydrophobic substituents (Figure 2.4). The sulfoamido group of compound 4 forms two hydrogen bonds with Leu-181 and Ala-182 by using the sulfonamido oxygen and the NH respectively [16].

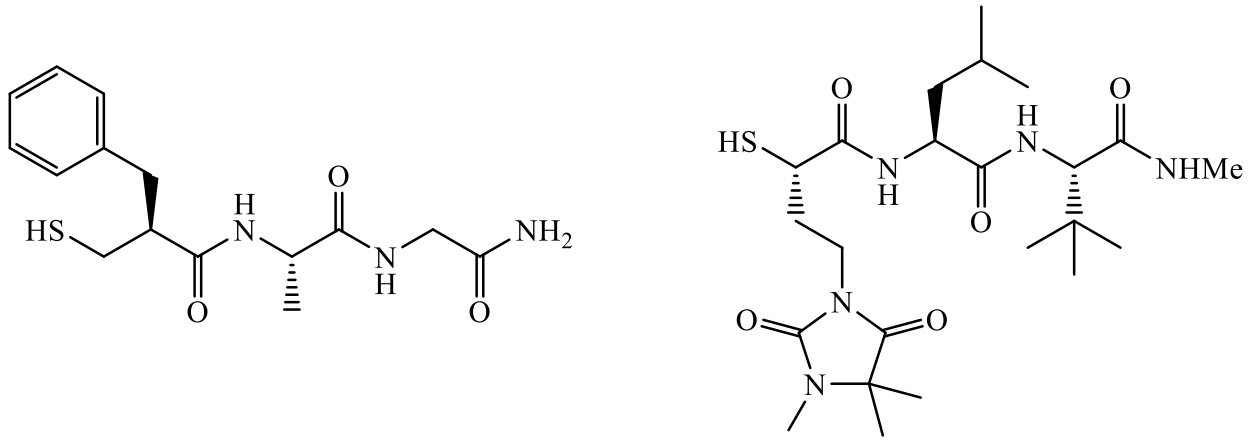
Figure 2.4 Structure of carboxylic-acid-based MMP inhibitors [16]



2) Thiol-based Inhibitors

Thiol-based MMP inhibitors utilize SH group as the ZBG. Though a five or six-membered ring can be formed if the inhibitor uses both its thiol group and carbonyl nearby to chelate the zinc (Figure 2.5), it only binds to zinc monodentately, so this type of inhibitor has a weaker binding affinity to zinc than the hydroxamates. Because thiol is easy to ionize, this class of MMP inhibitor is less potent than the carboxylates [16]. Two compounds from this class have entered clinical trial and one of them is compound 6, which is a strong MMP-8 and MMP-13, and it is being developed as an anticancer drug [16].

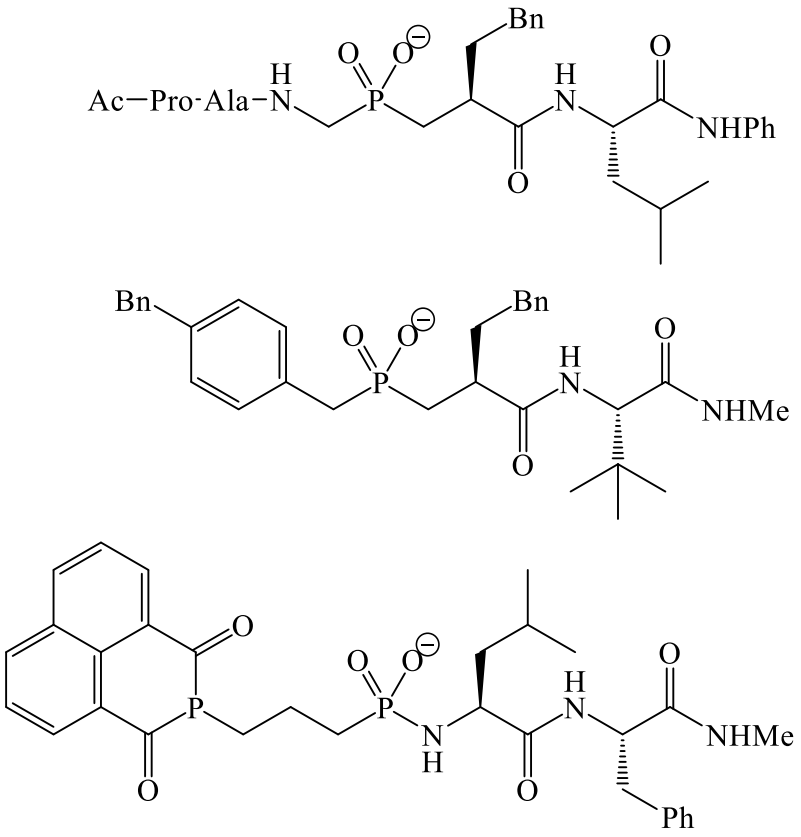
Figure 2.5 Structure of thiol-based MMP inhibitors [16]



3) Phosphorus-based Inhibitors

Either a phosphinic acid or a phosphonic acid group is present in the phosphorus-based inhibitors as the ZBG. Despite the initial excitement of its discovery, these compounds didn't exhibit comparable results to those of the hydroxamates [16]. Figure 2.6 shows the structure of some compounds of this class.

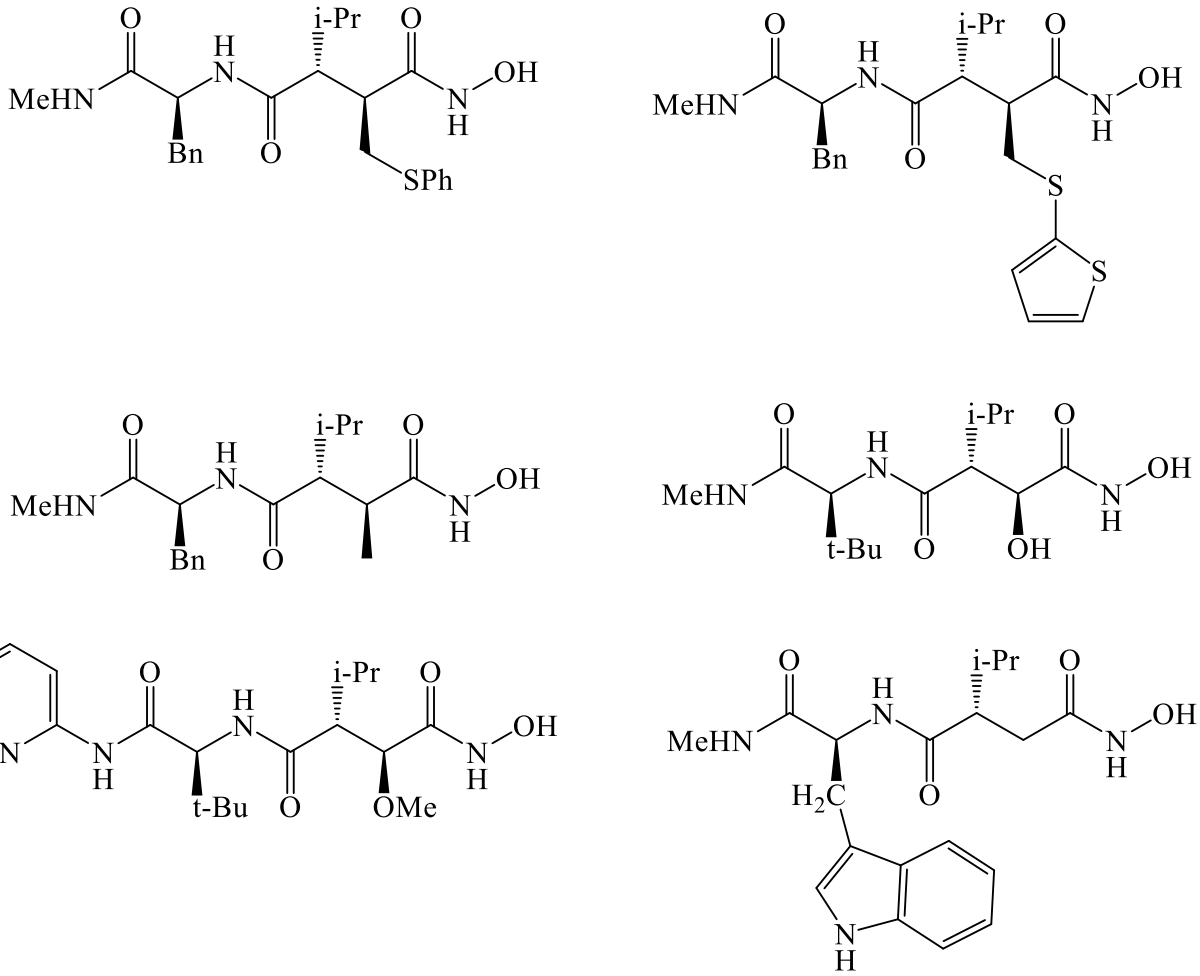
Figure 2.6 Structure of phosphorus-based MMP inhibitors [16]



4) Hydroxamate-based MMP Inhibitors

Hydroxamate-based MMP inhibitors are amongst the most widely studied inhibitors currently. The ZBG of the hydroxamates is the HONHCO functional group, the NH will be deprotonated and the carbonyl oxygen will bond to the zinc ion. The first generation of hydroxamates usually had a polypeptide backbone, which makes them metabolically labile (Figure 2.7) [54]. The broad spectrum of inhibition also causes serious side-effects and therefore the clinical use of hydroxamates as inhibitors against MMPs was abandoned.

Figure 2.7 Structure of hydroxamate-based MMP inhibitors [54]

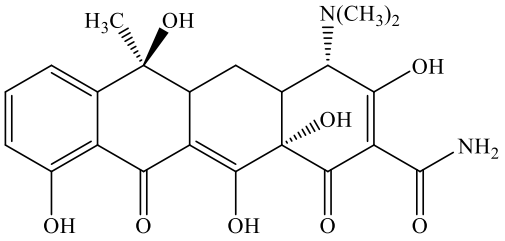


5) Chemically-modified Tetracyclines

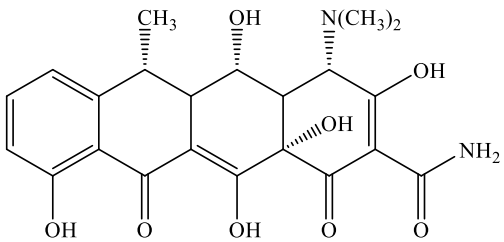
Tetracyclines have long been known as antibiotics, since their discovery by Duggar in 1948 from the soil organism *Strptomyces aureofaciens*. However, tetracyclines do have innate anti-MMP activity. In 1983, Golub *et al.* reported that chemically-modified tetracyclines (CMTs) could both inhibit collagenase activity in a diabetic rat model and regulate several other MMPs by a non-antimicrobial mechanism in tissue degradation and remodeling [5,6,21,54,55]. Therefore, a series of more than 30 CMTs were developed, lacking the dimethylamino group at C-4 position [20]. Doxycycline and its CMT derivatives CMTs-1, -3, -6, -7, -8 were found to inhibit bone

resorption *in vivo*, with CMT-3 as the superior one against purified collagenases from gingiva, osteoblasts and lung cancer cells [56-58]. Studies have also shown that TCs and CMTs not only inhibit MMP activity, but also inhibit the activation of MMPs at the proenzyme stage as occurs in MMP expression [58,59]. This led to the development of two FDA-approved drugs: Oracea® for chronic inflammatory skin disease and Periostat® for chronic inflammatory oral-bone-destructive periodontitis. Both of them are orally-administered forms of Doxycycline and are given at sub-antimicrobial dose levels. However, a significant side-effect of TCs and CMTs is increased photosensitivity particularly at higher doses, and this has limited their long-term use [60,61]. The structure of doxycycline and some CMTs are shown in Figure 2.8. The ZBG of this series of compounds is the 1-ketone-3-enol motif at the C-11, C-11a, C-12 positions.

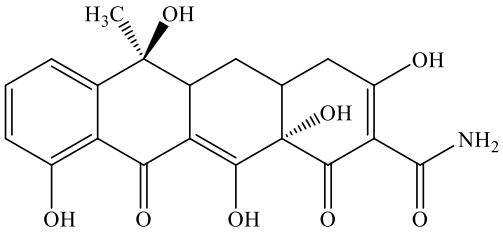
Figure 2.8 Structure of tetracycline, doxycycline and chemically-modified tetracyclines (CMTs)



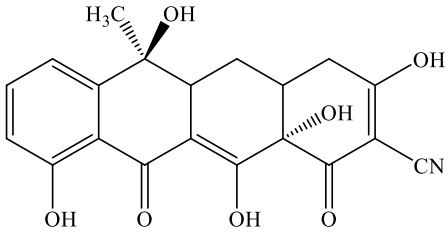
Tetracycline



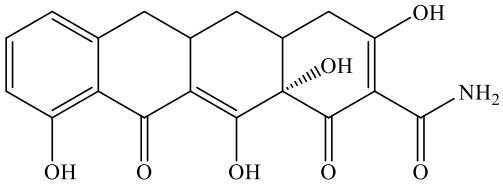
Doxycycline



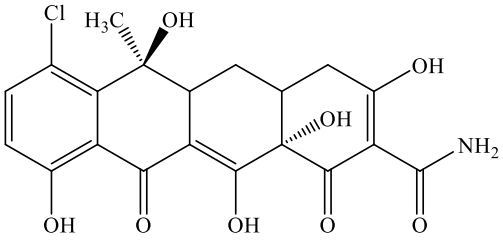
CMT-1



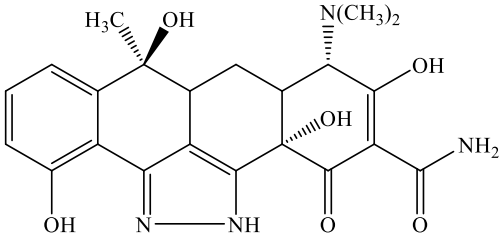
CMT-2



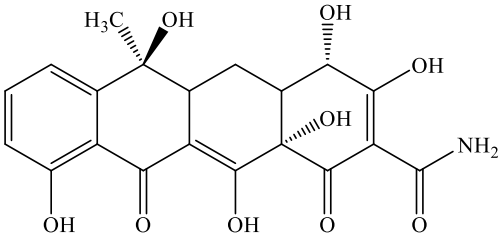
CMT-3



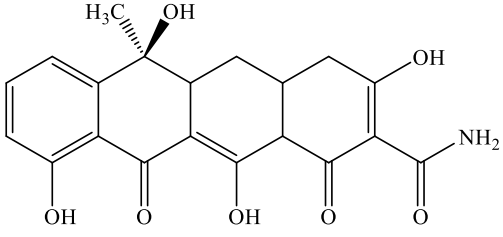
CMT-4



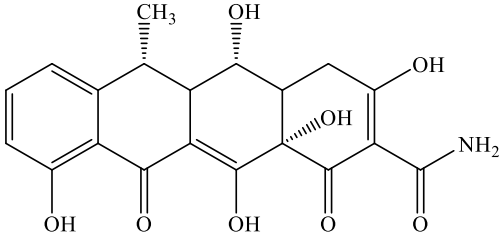
CMT-5



CMT-6



CMT-7



CMT-8

6) Chemically-modified Curcumins

Curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1E,6E-heptadiene-3,5-dione], with its mono-demethoxy and bis-demethoxy lower homologues, are all natural products found in turmeric. Turmeric is also known as "curry powder", and is used as a coloring agent and a therapeutic medication. Curcumin was first isolated by Vogel *et al*. in 1815, and its structure was confirmed by Lampe *et al*. in 1910 [62]. As a therapeutic reagent, curcumin exhibits a variety of activities including anti-cancer effect, anti-inflammatory activity, and anti-oxidant activity [63-68]. Curcumin also possesses the enolizable 1,3-diketo functionality similar to the TCs and is known to inhibit MMPs. Though curcumin is reasonably soluble in organic solvents including dimethylsufoxide, dimethylformamide, tetrahydrofuran, dichloromethane, and alcohols, it is extremely insoluble in water or physiological buffer [69], and this leads to poor absorption in animal models and human studies. Only 51.2ng/mL was found 4h in the serum after 12g of curcumin was orally administered [70] (Table 2.2).

Table 2.2 Serum concentration of curcumin by oral administration

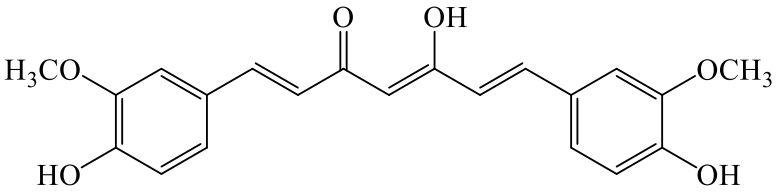
(Modified from references [70-73])

Species	Oral Dosage	Concentration in Serum
Rat	1g/kg	0.5μg/mL
Rat	2g/kg	1.35μg/mL
Human	2g	6.0ng/mL
Human	10g	50.5ng/mL

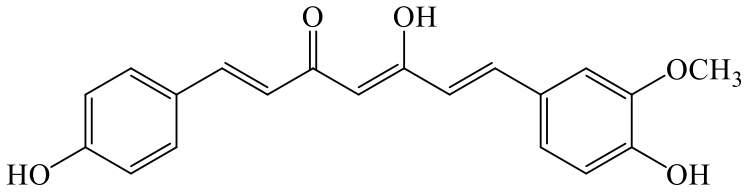
Human	12g	51.2ng/mL

To improve its water solubility, bioavailability as well as zinc-binding characteristic, research has indicated by the Johnson group to try to improve both activity and solubility. So far 23 chemically-modified curcumins (CMCs) have been synthesized and this work has been published [74]. The most potent one of the CMCs is CMC 2.24, which can inhibit a series of MMPs at low μM level (IC_{50} value ranges from 2.0±0.4 μM for MMP-12 to 69.8±2.0 μM for MMP-1) [74].

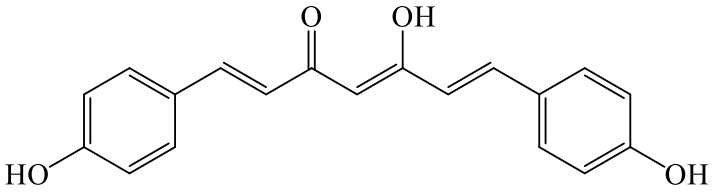
Figure 2.9 Structure of Curcumin, Demethoxycurcumin, bis-demethoxycurcumin, General Structure of Chemically-modified Curcumins and CMC 2.24



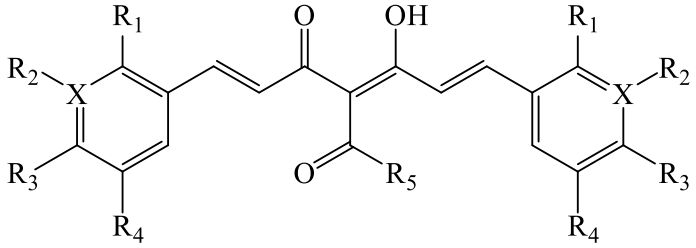
curcumin



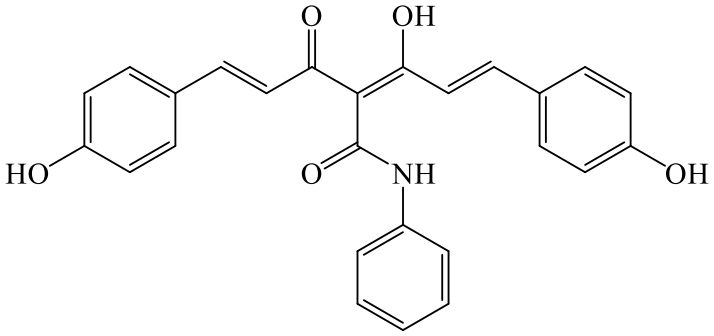
demethoxycurcumin



bis-demethoxycurcumin



general structure of chemically-modified curcumins



CMC 2.24

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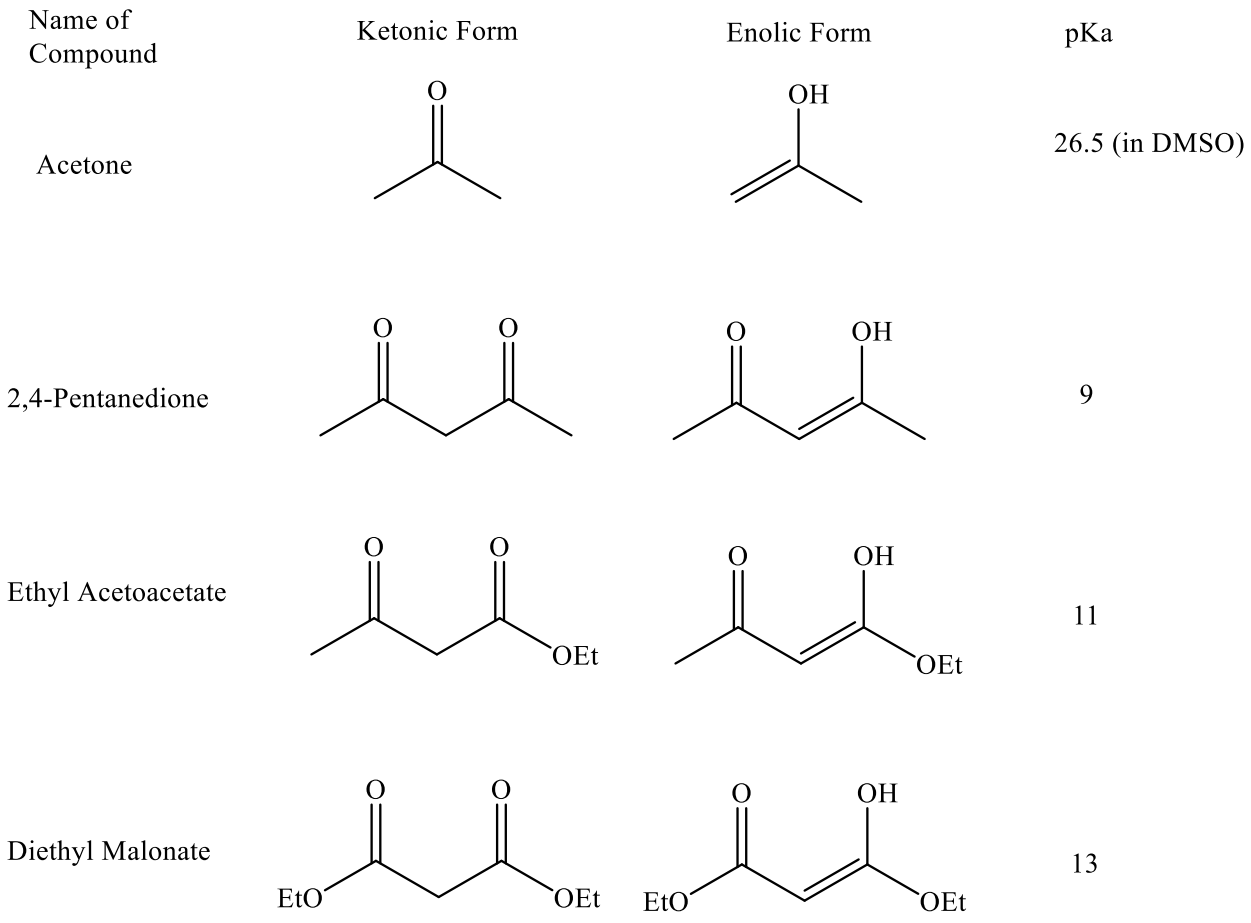
Chapter 3 Synthesis of Bis-aroylmethanes and Chemically-modified Curcumins and Preliminary Biological Screening of Bis-aroylmethanes against Matrix Metalloproteinase-9

A. Ketone-enol Tautomerization in 1,3-dicarbonyl Compounds

It has long been known that there is an equilibrium between aldehyde/ketone and its corresponding enol. One of the early investigators into this field was R. A. C. E. Erlenmeyer, who concluded in 1880 in a statement that was later known as "the Erlenmeyer Rule", which states that all alcohols in which the hydroxyl group is attached directly to a double-bonded carbon atom become aldehydes or ketones. This is true for most simple aldehydes and ketones. However, compounds with the 1,3-dicarbonyl motif are exceptions to this rule. Compounds with this functionality tend to favour the enolic form rather than the ketonic one. Three typical compounds of this kind, namely 2,4-pentanedione, ethyl acetoacetate and diethylmalonate, had been widely studied and used in organic synthesis. The major reasons for these compounds to favour the enolic form are: 1. Enolization can bring two separate double-bonds conjugated; 2. Enolization can help to form an intramolecular hydrogen-bond. Moreover, 1,3-dicarbonyl compounds have significant lower pKa values than those of other aldehydes/ketones which could not enolize. This acidic property of 1,3-dicarbonyl compounds is vital in MMP inhibition, since all previously disclosed MMP inhibitors possesses some degree of acidity. A fundamental idea in the current research project is to create 1,3-diketo compounds similar to curcumin but which are more acidic (to achieve better zinc binding). Some of the carbonyl compounds, with their physical data are listed in Figure 3.1.

Figure 3.1 pKa and Structure of the Ketonic and Enolic Form of Acetone, 2,4-pentanedione, Ethyl Acetoacetate and Diethyl Malonate



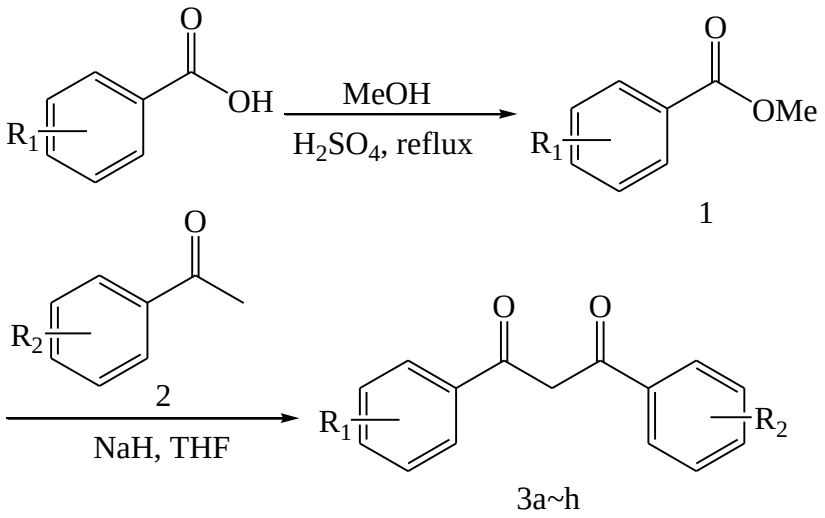
B. Synthesis of bis-aroyl Methanes

Bis-aroylmethanes (BAMs) are 1,3-dicarbonyl compounds. Its simplest one, bis-benzoylmethane is the diphenyl homologue of 2,4-pentanedione, which may also be called bis-acetylmethane. With a pKa value of 8.95 [1], bis-benzoylmethane is also fairly acidic. Since the physiological pH of serum is roughly 7.35~7.5, a small portion of bis-benzoylmethane and its more acidic derivative can ionize at this pH, and the ionized species can potentially be an MMP inhibitor.

1) General Synthetic Scheme

The general method to synthesize BAMs is outlined in Scheme 3.1 and starts with the preparation of corresponding methyl benzoates. Heat the benzoic acid in methanol with catalytic amount of sulfuric acid overnight, leads to the formation of methyl ester. The key reaction for the synthesis of the BAMs is a Claisen-type condensation. Sodium hydride is used as a base to deprotonate the α–hydrogen of the acetophenone, the subsequent enolate then attacks the carbonyl group of the methyl ester, replacing the methoxide by an addition-elimination mechanism. This is a rapid approach of the method that is used to generate BAMs. However, some functional groups need protection/deprotection, and the substituent at C-2 position of BAMs can only be installed after the main BAM framework had been established. This of course necessitates further steps. The synthetic scheme is shown below and described in the following section 2, 3, and 4.

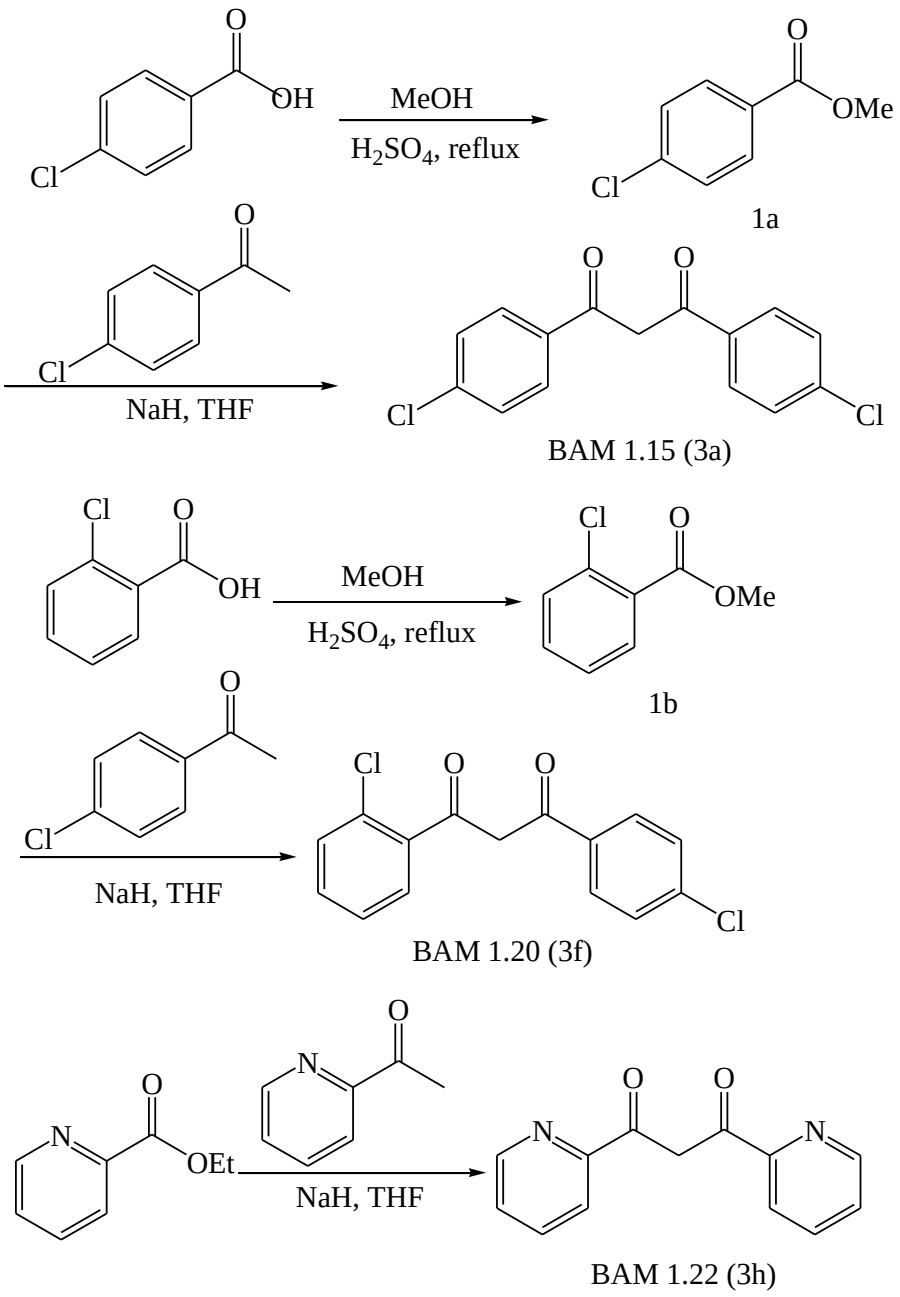
Scheme 3.1 General synthesis of BAMs



2) Synthesis of BAM 1.15, 1.20, 1.22

The syntheses of BAM 1.15 and 1.20 began with the convertion of the 2- or 4-chlorobenzoic acid to its corresponding methyl ester, which occurred smoothly under reflux condition in methanol overnight catalyzed by sulfuric acid. The methyl ester then is allowed to react with 4-chloroacetophenone in a Claisen-type condensation to afford BAM 1.15 (**3a**) and 1.20 (**3f**). BAM 1.22 (**3h**) however, is directly obtained by condensing ethyl picolinate and 2-acetylpyridine. The syntheses are outlined in Scheme 3.2.

Scheme 3.2 Synthesis of BAM 1.15, 1.20, 1.22

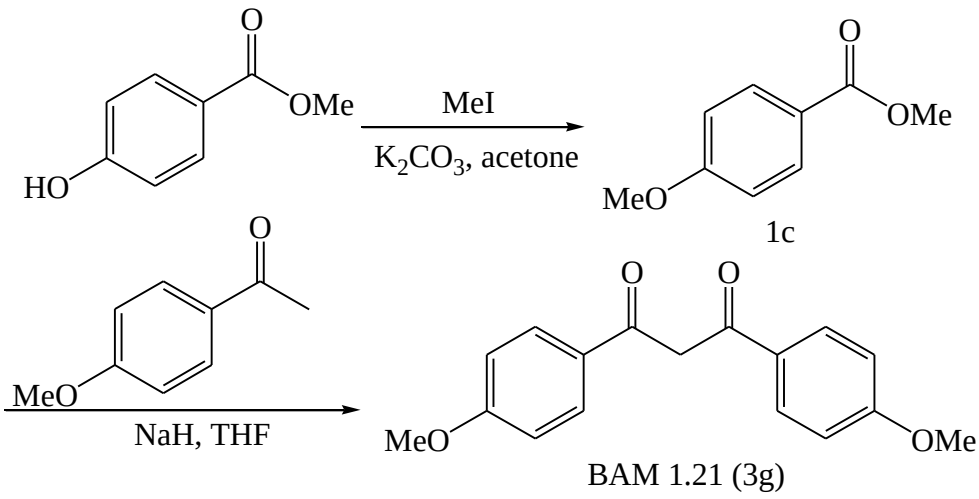


3) Synthesis of BAM 1.21

The synthesis of BAM 1.21 (**3g**), namely bis-(4-methoxybenzoyl)methane began with the methylation of 4-hydroxybenzoic acid methyl ester with methyl iodide under basic condition.

The resulting 4-methoxybenzoic acid methyl ester can condense with 4-methoxyacetophenone to yield the title compound. The synthetic scheme is shown in Scheme 3.3.

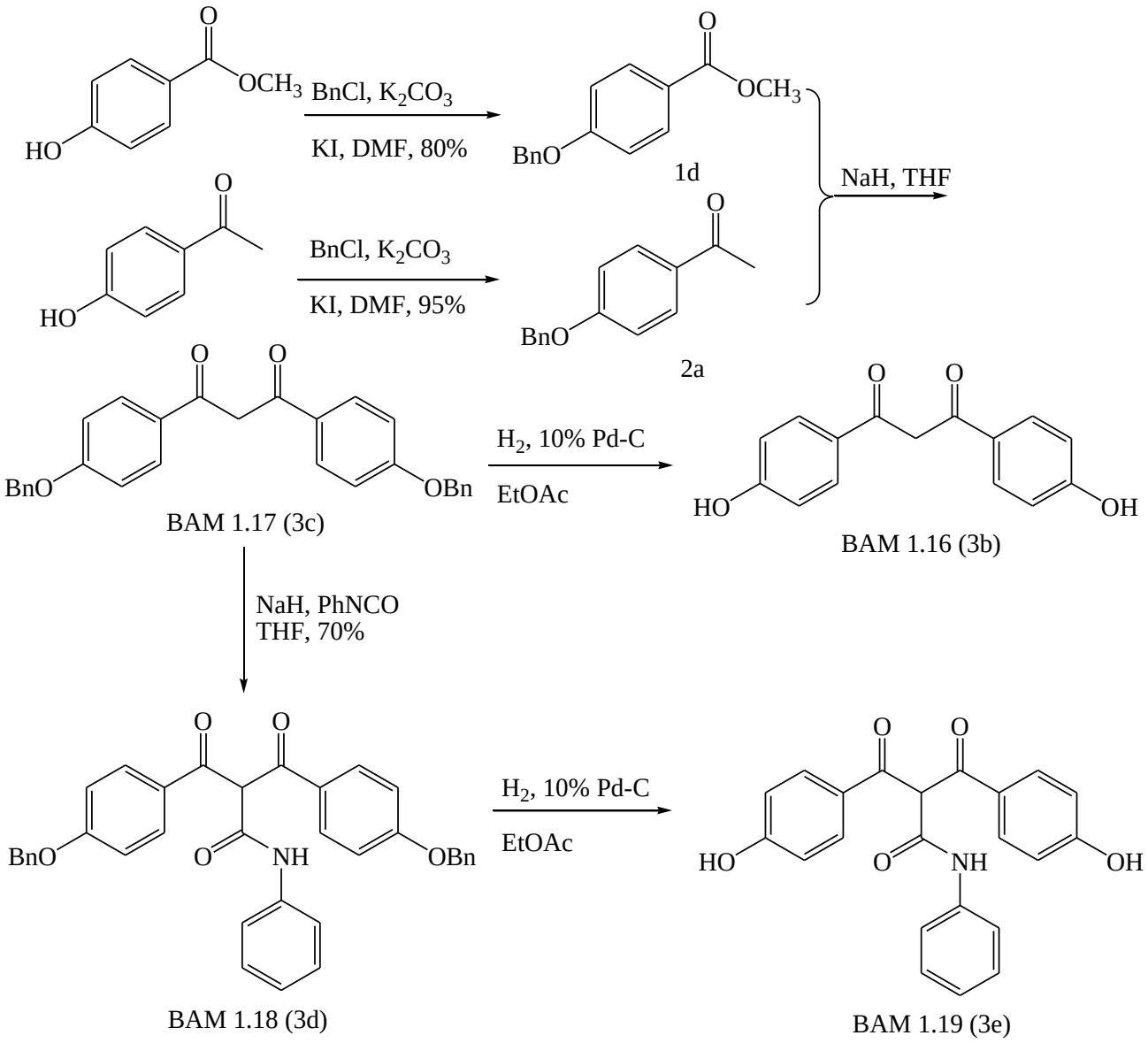
Scheme 3.3 Synthesis of BAM 1.21



4) Synthesis of BAM 1.16, 1.17, 1.18, 1.19

The syntheses of BAMs 1.16~1.19 began with the protection of the hydroxyl group in 4-hydroxyacetophenone and 4-hydroxybenzoic acid methyl ester, using benzyl chloride in DMF, catalyzed by potassium iodide. The benzylated acetophenone and benzoic acid methyl ester were then allowed to condense to form BAM 1.17 (3c). From BAM 1.17 three other BAMs were prepared. Deprotecting the benzyl group of BAM 1.17 by hydrogenation led to the formation of BAM 1.16 (3b). BAM 1.18 was obtained by deprotonating BAM 1.17 by NaH to give the corresponding enolate. The subsequent enolate was then allowed to reacte with phenyl isocyanate to obtain BAM 1.18 (3d) [2]. Hydrogenation of BAM 1.18 gave BAM 1.19 (3e). The syntheses of these four BAM compounds are outlined in Scheme 3.4.

Scheme 3.4 Synthesis of BAMs 1.16, 1.17, 1.18, 1.19



C. Preliminary Biological Screening of BAMs against MMP-9

1) Experimental Setup

The initial biological screening of BAMs involves a procedure to measure their IC_{50} values. This is the most elementary of methods. In our experiments, we used a synthetic polypeptide that can

be cleaved by MMPs. The MMP chosen for our studies was MMP-9, human neutrophil monomer, purchased from Calbiochem, EMD Biosciences, Inc. (La Jolla, CA), and the polypeptide substrate was Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 fluorogenic peptide substrate IX, purchased from R&D Systems, Inc. (Minneapolis, MN) [3,4]. [Mca is (Methoxycoumarin-4-yl)acetyl; Dpa is N-3-(2, 4-Dinitrophenyl)-L-2,3-diaminopropionyl]. This substrate is linked to a fluorescent probe. Once cleaved by an MMP, the fluorescent group will be released and the fluorescence signal increases dramatically. The fluorescence signal is measured at the excitation and the emission wavelengths of 320nm and 405nm respectively.

Stock solutions of 1,10-phenanthroline, curcumin and curcumin analogues were prepared in DMSO at concentrations of 0.1, 0.5, 1, 5, 10, 25 and 50mM (for BAM 1.17, it is 0.1, 0.2, 0.5, 1, 2, 4, 8mM). The substrate and the enzyme were diluted 100 fold with solution containing 1mM $CaCl_2$, 0.2M NaCl and 50mM Tris/HCl (pH=7.6) buffer. 50μL of substrate solution was added first to the 96-well plate, followed by the addition of different concentrations of DMSO solution of inhibitor (1μL) and enzyme solution (50μL) to a final reaction volume now containing 1% DMSO. The fluorescence signal of the solution of the substrate and inhibitors at different concentration (without MMP-9) was measured as the background. The test solution mixed with MMP-9 and substrate was set as the peak value. The whole mixture was incubated at 37^{o}C for 2h, and Fluoro Count (Packard Instrument Co., CT) was used to measure the increase in fluorescence as the substrate was cleaved. The increase in fluorescence of the test solution of MMP-9, substrate and inhibitor was then compared with the peak value and the background of its corresponding concentration to calculate the percentage of MMP being inhibited. Concentration of total inhibition was not measured.

2) Results and Discussion

Out of the seven BAMs tested, BAM 1.15 and BAM 1.18 showed an IC_{50} value of greater than 100μM. BAM 1.17 had an IC_{50} value of greater than 20μM, and the inhibitory effect at higher concentration was not tested due to solubility problem. The inhibitory assays of BAM 1.16, BAM 1.19, BAM 1.20, BAM 1.21 compared with the reference compound, namely 1,10-phenathroline, are illustrated below in Figure 3.2a~3.2c.

Figure 3.2a Test 1 in MMP-9 Inhibition Assay

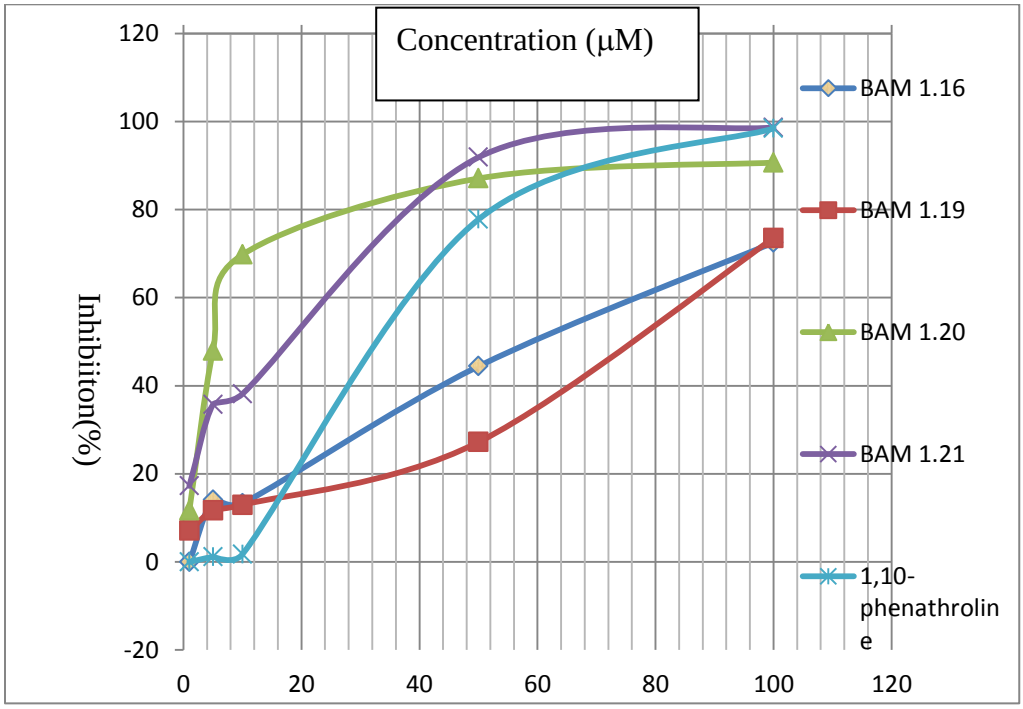


Figure 3.2b Test 2 in MMP-9 Inhibition Assay

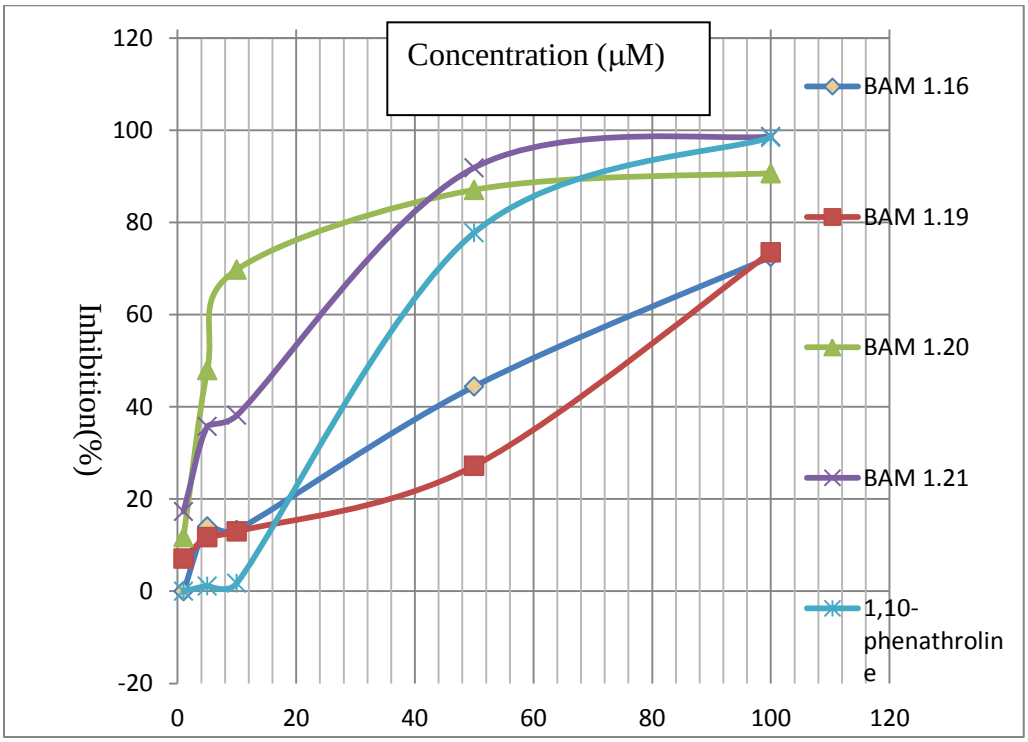
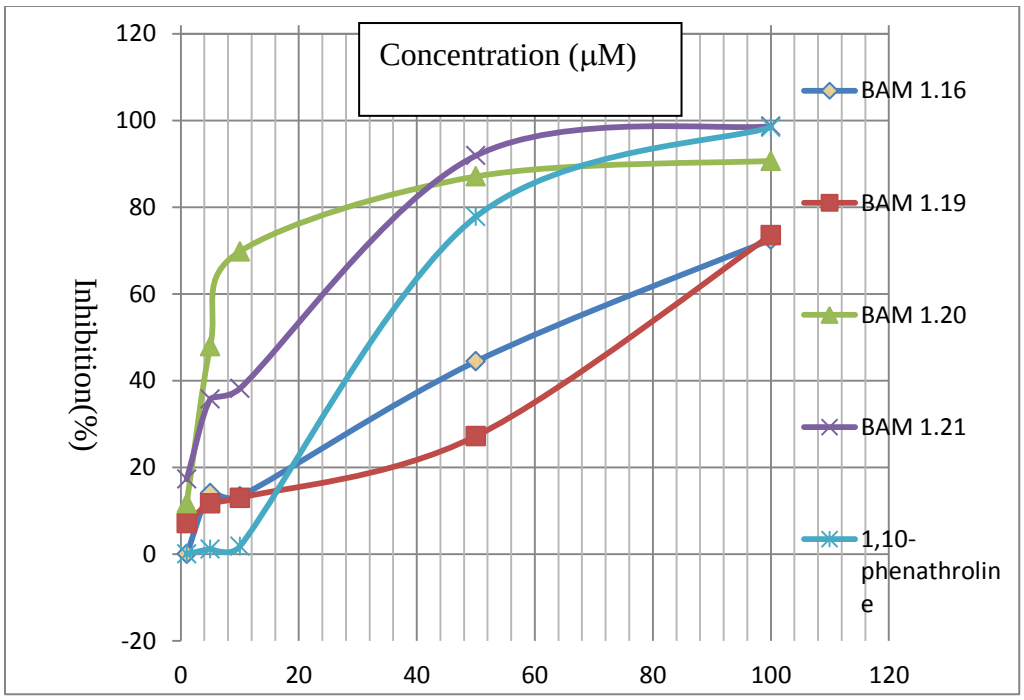


Figure 3.2c Test 3 in MMP-9 Inhibition Assay



The structures of the BAMs are summarized in Figure 3.3 and Table 3.1 respectively.

Figure 3.3 Enolic/Ketonic Tautomerism of BAMs

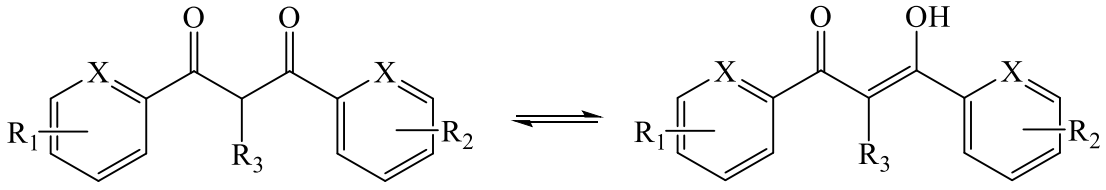


Table 3.1 Structure of BAMs Synthesized

BAM	R_1	R_2	R_3	X	Enol Pencentage
BAM 1.15	4-Cl	4-Cl	-H	CH	100
BAM 1.16	4-OH	4-OH	-H	CH	74
BAM 1.17	4-$PhCH_2O$	4-$PhCH_2O$	-H	CH	100
BAM 1.18	4-$PHCH_2O$	4-$PhCH_2O$	-CONHPh	CH	0
BAM 1.19	4-OH	4-OH	-CONHPh	CH	0
BAM 1.20	2-Cl	4-Cl	-H	CH	100
BAM 1.21	4-OCH_3	4-OCH_3	-H	CH	100
BAM 1.22	-H	-H	-H	N	87

The IC_{50} value of the control compound (1,10-phenathroline) and BAMs 1.16, 1.19, 1.20, 1.21 are summarized in Table 3.2.

Table 3.2 IC_{50} of 1,10-phenathroline and Selected BAMs against MMP-9

		1,10-Phen	BAM 1.16	BAM 1.19	BAM 1.20	BAM 1.21
Test 1	$IC_{50}(\mu M)$	22.8	68.7	72.4	5.1	20.9
Test 2	$IC_{50}(\mu M)$	32.7	59	76.2	5.3	17.9
Test 3	$IC_{50}(\mu M)$	24.4	67.7	72.1	5.1	16.5
	Ave.	26.6	65.1	73.6	5.2	18.4
	S.D.	5.3	5.3	2.3	0.1	2.2
	S.E.M.	3.1	3.1	1.3	0.1	1.3

From Table 3.1, we can conclude that: when there is no substitution at the C-2 position, BAM compounds tend to favor the enolic form in the ketone/enol equilibrium. C-2 substituted BAMs tend to maintain the ketonic form due to allylic strain which overcomes conjugation and intramolecular hydrogen bonding. For BAM 1.16, the relatively low enolic percentage (74%) is probably because of the intermolecular hydrogen bonding between the hydroxyl group and the carbonyl, which may prevent the carbonyl group to receive the hydrogen bonding from the enolic hydrogen, thus disfavoring the enolic form. In BAM 1.22 however, the 2-pyridyl group may serve as a strong electron-withdrawing group to diminish the electron density on the

carbonyl oxygen, and this may prevent the hydrogen bond formation, since oxygen acts as an electron donor in forming the intramolecular hydrogen bond.

Comparing the IC_{50} value, BAM 1.16 is has a lower IC_{50} than its C-2 substituted derivative, BAM 1.19. Also BAM 1.19 which has a C-2 substituent has a higher IC_{50} than its non-substituted analogue BAM 1.16. Thus, C-2 substitution may not be favourable in increasing the inhibitory effect of the BAM series. Comparing BAM 1.15, 1.16 and 1.21, it seems that the compounds with electron-donating groups are more potent than the one with electron-withdrawing groups. The most potent is the unsymmetrical compound. The 2-position of the chlorine substituent on one phenyl ring decreases the IC_{50} to lower than $^1/_{20}$ of the bis-4-position-substituted analogue. Though it is clear that the positioning of chlorine substitute can make a drastic difference on the inhibitory effect, the exact reason of its effect on inhibition, is still unknown.

D. Synthesis of Chemically-modified Curcumins

1) Project Background and Target Design

Curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], as mentioned earlier, is another 1,3-dicarbonyl compound and a bis-vinylene homologue of BAMs. It was isolated in 1815 by Vogel *et al*. from the perennial herb that grows in Asia called *Curcuma longa L*. Its structure was first illucidated by Lampe *et al*. in 1910 [5]. Since then, numerous papers have been published to deal with the chemical method of its synthesis. The first synthesis was done by Lampe et al. in 1918, which contained an 8-step synthesis starting from vanillin [6]. Almost 2 decades later, Pavolini et al. carried out a one-step synthesis using 2,4-pentanedione, vanillin and boron oxide (2:1:2) over a free flame for 30 min, with a low yield of 10% [7]. In 1962, Pabon et al. published his work of curcumin synthesis also by using the same reactants as

those of Pavolini et al [8], but with different stoichiometry [2,4-pentanedione:boron oxide=1:0.7] (Scheme 3.5). In Pabon's paper, a deep-coloured boron-complex was formed from 2,4-pentanedione, vanillin, tri-n-butyl borate and boron oxide under the catalysis of 1-butylamine in ethyl acetate. The intermediate boron-complex can be decomposed in 0.4N HCl at 60^{o}C to afford the desired product. In this procedure, boron oxide was used to prevent Knoevenagel condensation at C-3 position because boron oxide can react with 2,4-pentanedione to inactivate the C-3 position with regard to condensation. Four equivalents of $(n\text{-}BuO)_3B$ (tri-n-butyl borate) is with respect to the amount of 2,4-pentanedione. Although it was found that tri-n-butyl borate may assist in dissolving the reactants, especially the boron species, the exact role of this component is still a mystery. Catalytic amount of a mild base like 1-butylamine or piperidine is also necessary to obtain a satisfactory synthesis. The results of Pabon's work are summarized in Table 3.3 [8].

Scheme 3.5 Pabon's Method to Synthesize Curcumin Analogues [8]

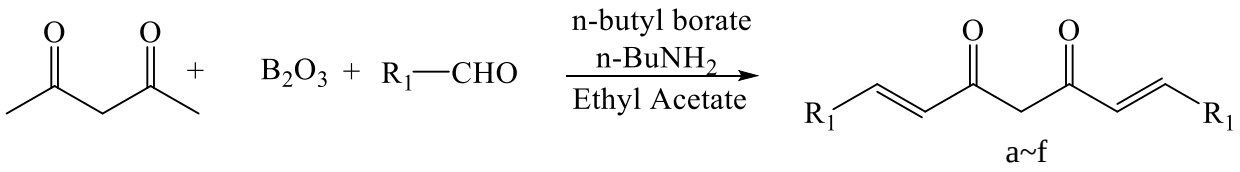


Table 3.3 Yields of Curcumin Analogues Synthesized by Pabon [8]

Compound	R_1	Yield(%)
A	4-hydroxy-3-methoxyphenyl	73
B	4-dimethylaminophenyl	36
C	3-nitrophenyl	25

D	4-methoxyphenyl	57
E	2-furyl	8
F	phenyl	23

It was found out that a thick paste was formed when 2,4-pentanedione and boron oxide were heated together. Pabon proposed a structure of this intermediate that indicated that the boron was chelated by two 2,4-pentanedionate, and this chelated boron ion was bonded to another anion (Figure 3.4). To confirm the structure of this intermediate, a former member of the Johnson group, Mr. Maro Kariya heated 2,4-pentanedione and boron oxide in DMF/benzene and a highly crystalline complex. The structure of this crystallized boron-complex was shown in Figure. In it, three boron atoms and three oxygen atoms form a 6-membered ring. Two boron atoms are chelated by 2,4-pentanedionate, whereas the 3rd boron atom is covalently bonded to a hydroxyl group. The hydroxyl group hydrogen is bonded to the oxygen atom of a DMF molecule via a hydrogen bond (Figure 3.5). It is from the crystal structure we calculated that the ideal stoichiometry between 2,4-pentanedione and boron oxide is 1:0.75.

Figure 3.4 Structure of Boron-complex Proposed by Pabon (left) and Kariya's work (right)

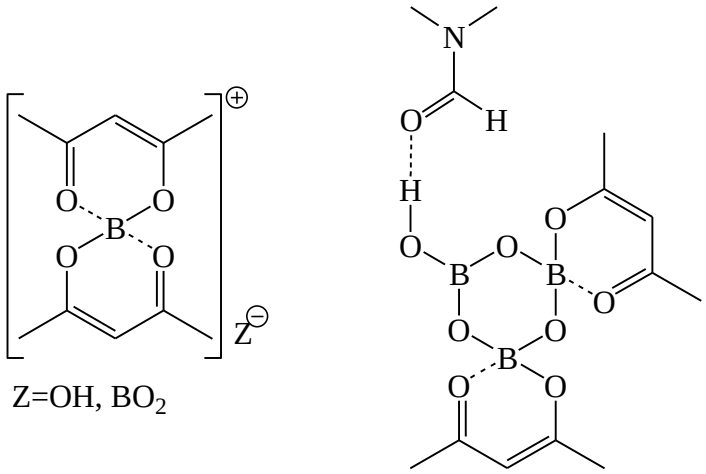
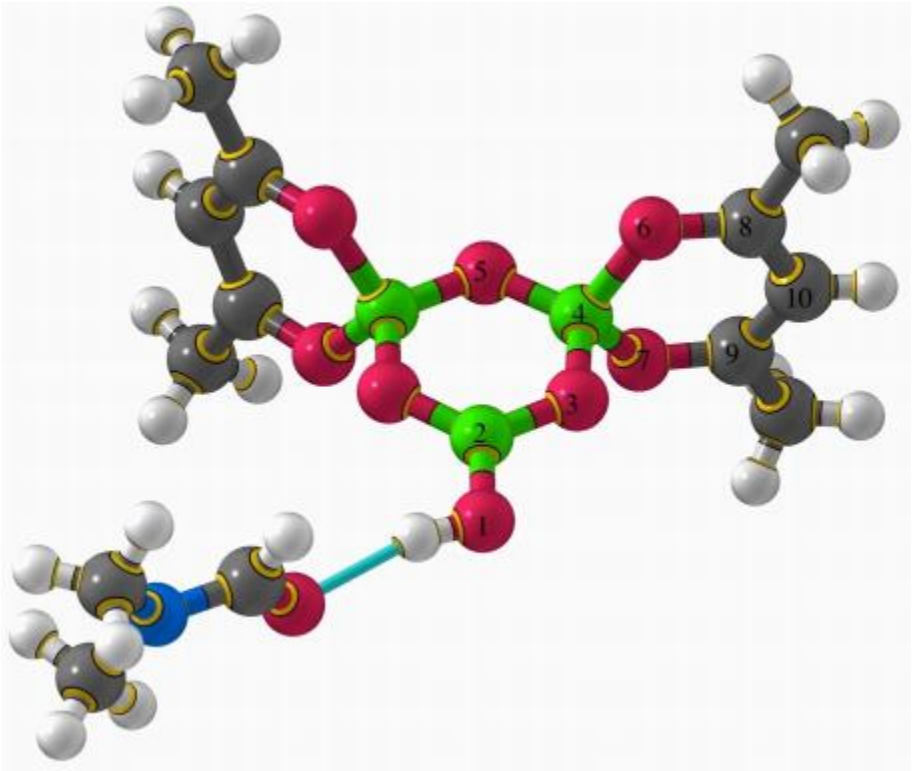


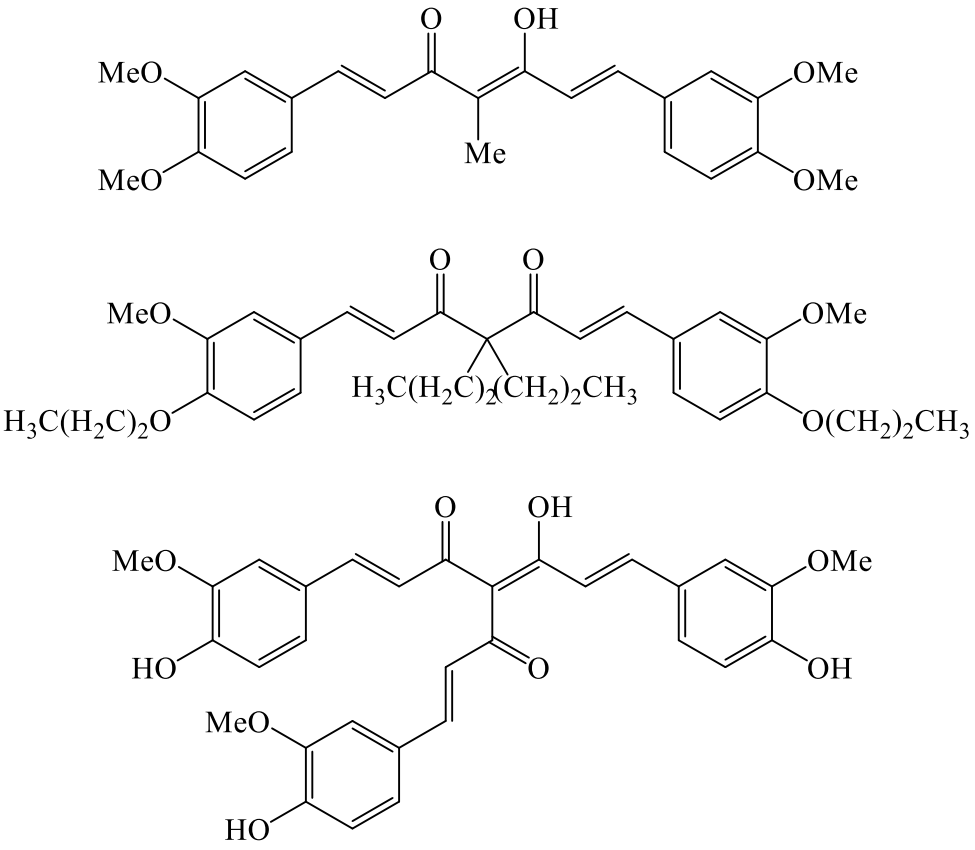
Figure 3.5 X-ray Crystallography of the Structure of Kariya's Boron-complex



In Pabon's work, only unsubstituted 2,4-pentanedione was used, therefore the curcumins synthesized didn't have a substituent at C-4 position. In 1980, Matthes et al. reported a synthesis

of 4-cinnamoyl-substituted curcumin analogues [9], and Ishida et al. published a 4-alkyl-substituted curcumin [10] whereas Lin et al. reported a synthesis of 4,4-disubstituted curcumin derivative [11] (Figure 3.6). Since then, a large number of curcumin analogues have been synthesized as potential agents against tumour cells.

Figure 3.6 Structure of 4-alkyl-substituted, 4,4-disubstituted and 4-cinnamoyl-substituted Curcumin Derivatives [9,10,11]

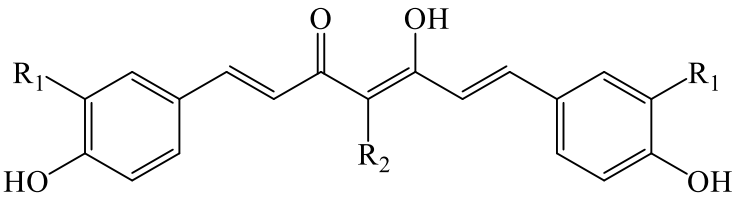


It is mentioned earlier that all synthesized MMP inhibitors possess some degree of acidity, which is vital for Zn-binding. Hypothetically, introducing one more EWG between the two carbonyl groups on the curcumin backbone may stabilize the negative anion, thus making the compound more acidic. According to Bernabé-Pineda *et al* [12], curcumin has three acidic hydrogens and

the pKa values are 8.38, 9.88, and 10.51. Since the physiological pH of serum is between 7.35 and 7.5, meaning only a small portion of curcumin can ionize and bind to the zinc ion at this pH level, therefore one more EWG can possibly lower the pKa of the compound, thus making it easier to ionize.

With this in mind, a former member of the Johnson Group, Dr. Yu Zhang published the synthesis and biological screening results of twenty-three chemically-modified curcumins (CMCs) [13]. These CMCs were synthesized in a short and straight-forward route by a modified Pabon reaction. In contrast to the 2-substituted BAMs, 4-substituted CMCs exclusively adopt enolic form, while the 2-substituted BAMs are entirely ketonic. It is because the bis-vinylene group in the backbone of CMCs provide enough space for the compound to be planar and to enolize. Of the twenty-three CMCs, four CMCs, namely CMC 2.5 , CMC 2.14 , CMC 2.23 , CMC 2.24 stand out from the other analogues in their inhibitory effect against MMPs in vitro [13] (structures of curcumin and four CMCs are shown in Figure 3.7). CMC 2.24 is the most potent compound and shows low-μM IC_{50} value against a range of MMPs (results summarized in Table 3.4).

Figure 3.7 Structure of Selected CMCs [13]



Curcumin: R_1=-OMe, R_2=-H
CMC 2.5: R_1=-OMe, R_2=-COOMe
CMC 2.14: R_1=-H, R_2=-COOMe
CMC 2.23: R_1=-OMe, R_2=-CONHPh
CMC 2.24: R_1=-H, R_2=-CONHPh

Table 3.4 IC_{50} of Selected CMCs against a Series of MMPs [13]

MMPs	1,10-phenathroline	curcumin	CMC 2.5	CMC 2.14	CMC 2.23	CMC 2.24
MMP-1	42.0±1.1	85.8±1.8	74.0±3.5	76.3±6.5	68.0±3.2	69.8±2.0
MMP-8	31.3±0.5	6.8±1.0	30.8±1.5	20.0±2.0	2.5±0.3	4.5±0.5
MMP-13	50.0±10.4	3.7±0.3	28.3±4.4	26.7±1.7	3.3±0.3	2.7±0.7
MMP-2	73.8±1.0	5.0±0.7	25.3±1.3	23.8±0.9	6.3±0.9	4.8±0.5
MMP-9	45.0±12.6	30.0±2.9	55.0±17.3	43.3±4.4	8.7±0.7	8.0±0.6
MMP-3	77.0±3.2	4.7±0.8	32.5±2.8	28.3±1.0	5.3±0.7	2.9±0.4
MMP-7	193.8±8.8	51.8±2.5	48.8±0.5	57.5±4.6	21.5±1.0	5.0±0.7
MMP-12	29.5±1.3	2.6±0.2	27.8±1.7	5.3±0.3	4.5±0.5	2.0±0.4
MMP-14	43.8±4.2	29.5±3.2	48.5±4.3	40.0±8.4	41.3±4.9	15.3±3.1

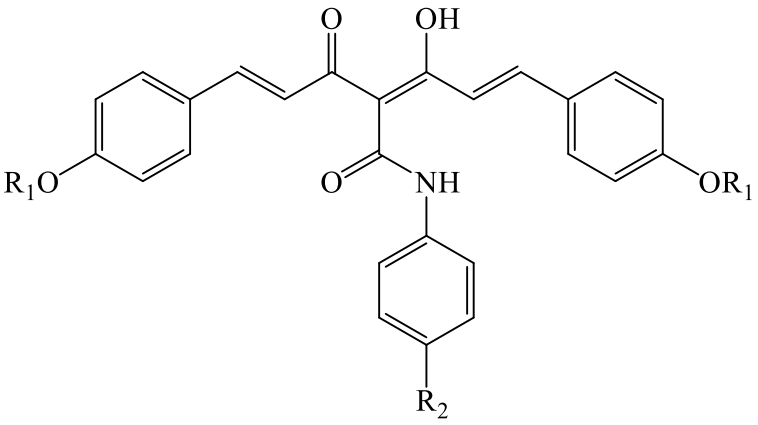
A further study into the pKa and zinc-binding affinity of curcumin, CMC 2.5 and CMC 2.24 was also done by Zhang *et al* [14]. It shows that introducing one more EWG at C-4 position of curcumin can indeed lower the pKa of the compound, just as previously hypothesized. However, the EWG can both increase and decrease the dissociation constants. Up to now, best results observed are associated with compounds with 4-phenylaminocarbonyl substituents. The pKa and dissociation constants of inhibitor-zinc complex are listed in Table 3.5.

Table 3.5 pKa and Dissociation Constants of Inhibitor-zinc Complex [12,14]

	pKa by Bernabé-Pineda *et al*.	pKa by Zhang *et al*.	Dissociation Constants of Inhibitor-zinc Complex (μM)
Curcumin	8.38, 9.88, 10.51	8.41, 9.94, 11.12	1385 ± 89
CMC 2.5	N/A	6.50, 8.82	1880 ± 68
CMC 2.24	N/A	6.98, 8.40, 9.80	765 ± 20

Furthermore, unpublished data shows that CMC 2.26 has a slightly better inhibitory profile than CMC 2.24, while CMC 2.25 is slightly less active than CMC 2.24. Therefore, we designed a compound, which was later termed CMC 2.34, as the new synthetic target. It is possible that acetates can be hydrolyzed in the human system, and some prodrugs are acetates of their active drug form. Therefore the di-acetate of CMC 2.24, which is also called CMC 2.32, and CMC 2.33, the tri-acetate of CMC 2.34, seemed also worth synthesizing (Figure 3.8).

Figure 3.8 Structure of CMCs 2.24, 2.25, 2.26, 2.32, 2.33, 2.34

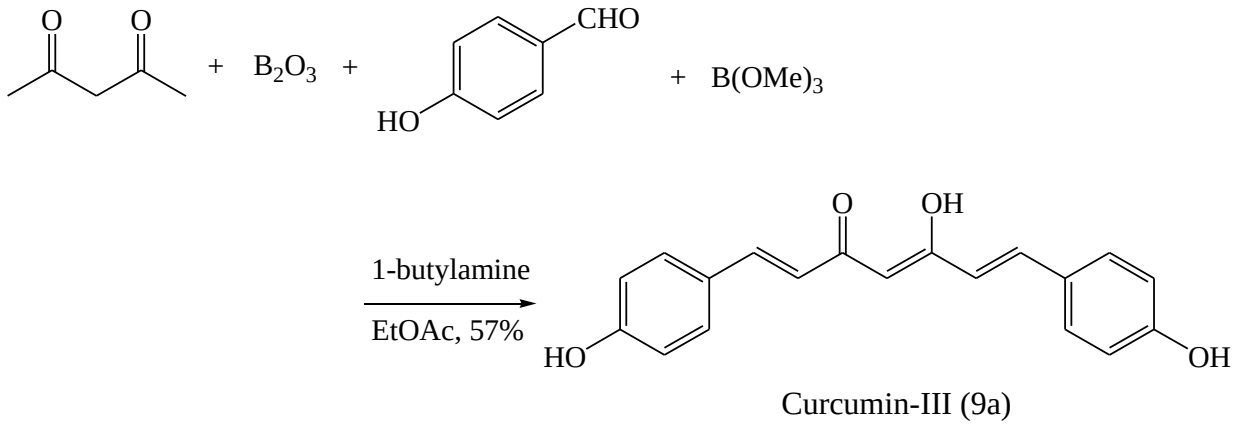


CMC 2.24: R_1=H, R_2=H
CMC 2.25: R_1=H, R_2=Cl
CMC 2.26: R_1=H, R_2=OMe
CMC 2.32: R_1=Ac, R_2=H
CMC 2.33: R_1=Ac, R_2=OAc
CMC 2.34: R_1=H, R_2=OH

2) Synthesis of Curcumin-III

Curcumin-3 (**9a**) was not mentioned in Pabon's work, so as a model to test the repeatability of the Pabon reaction, the synthesis of curcumin-III was accrued out by using 2,4-pentanedione, boron oxide, 4-hydroxybenzaldehyde, trimethyl borate and 1-butylamine and obtained the desired product in 57% yield (Scheme 3.6).

Scheme 3.6 Synthesis of Curcumin-III

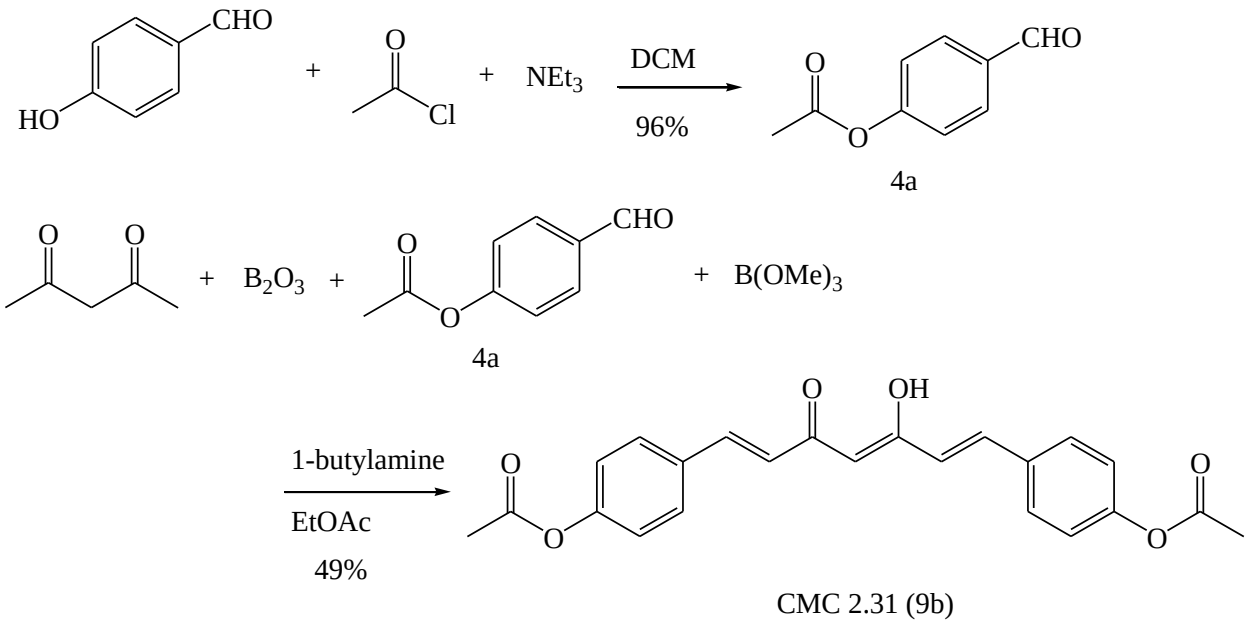


Curcumin-III (9a)

3) Synthesis of CMC 2.31

CMC 2.31 (**9b**), the di-acetate of curcumin-3, was not made by Pabon's method, but by acetylation of curcumin-3 [15]. Since both CMC 2.32 and CMC 2.33 share the same backbone of CMC 2.31, therefore it seemed worthwhile to synthesize this compound as an important intermediate for the synthesis of other 2 CMCs. The synthesis of CMC 2.31 began with the acetylation of 4-hydroxybenzaldehyde with acetyl chloride in 96% yield. Then 4-acetoxybenzaldehyde (**4a**) was used in the subsequent Pabon Reaction to produce the desired material (**9b**) in 49% yield (Scheme 3.7).

Scheme 3.7 Synthesis of CMC 2.31



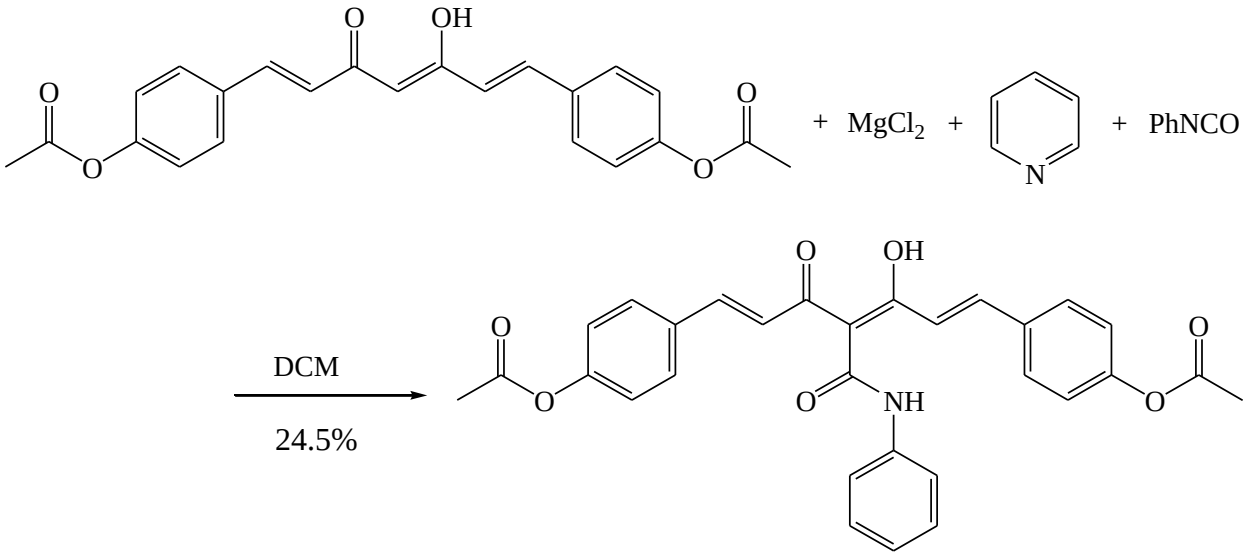
CMC 2.31 (9b)

4) Synthesis of CMC 2.32

With CMC 2.31 at hand, CMC 2.32 (**9c**) was synthesized by the well-established method according to Zhang et al. used [13]. Pyridine was used to deprotonate the enolic proton, and the

chelated magnesium species becomes the nucleophile, which is to attack the phenyl isocyanate. The desired molecule CMC 2.32 (**9c**) was obtained in 24.5% isolated yield (Scheme 3.8).

Scheme 3.8 Synthesis of CMC 2.32

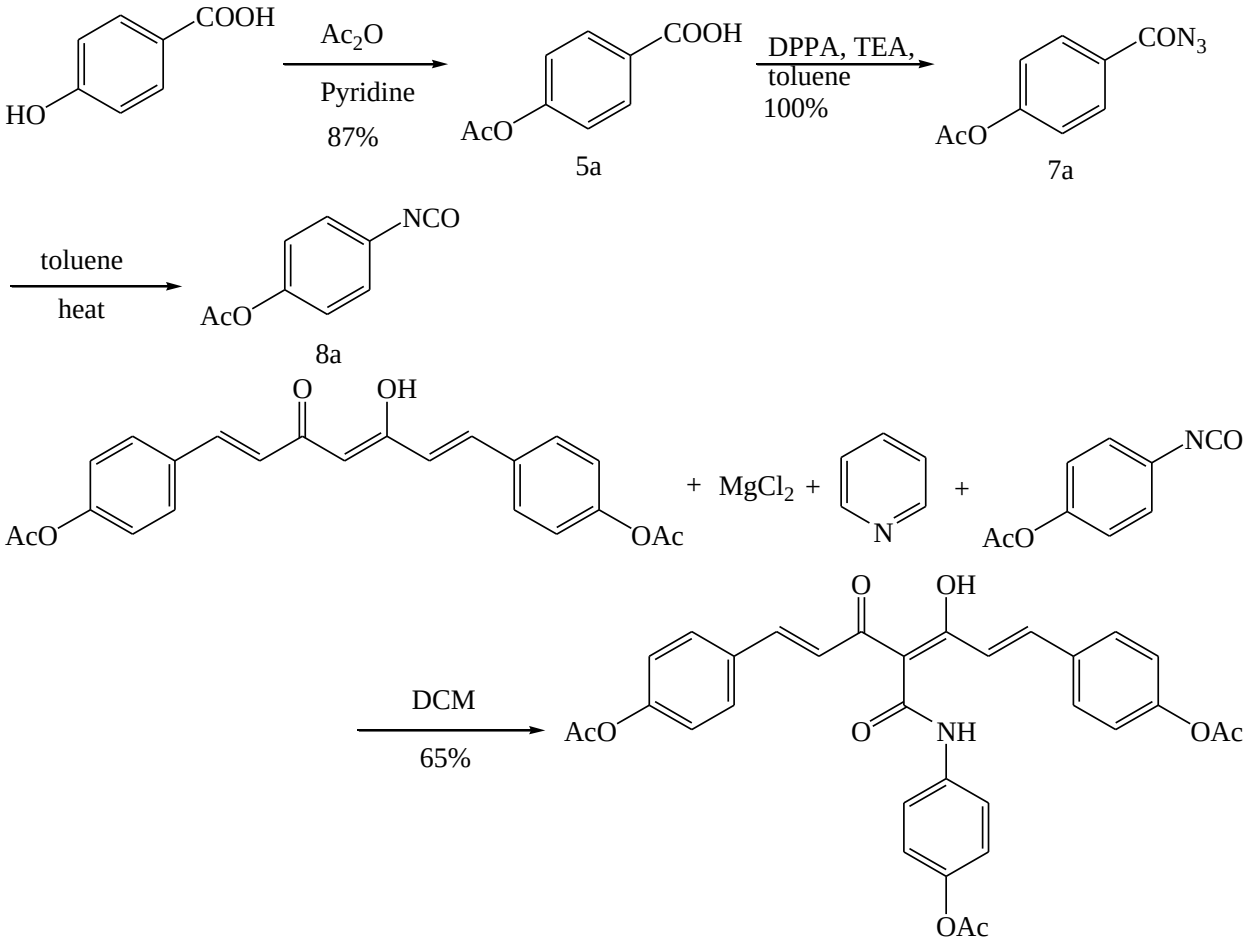


CMC 2.32 (9c)

5) Synthesis of CMC 2.33

CMC 2.33 (**9d**) was prepared in a way similar to that of CMC 2.32. However, the required isocyanate was not available commercially and therefore was synthesized de novo. Acetylation of 4-hydroxybenzoic acid with acetyl anhydride catalyzed by H_2SO_4 was completed in 83% yield. The acetylated benzoic acid (**5a**) was then quantitatively converted to its corresponding acyl chloride (**6a**), followed by substitution of the chlorine by the azide group using diphenylphosphoryl azide (DPPA) and triethylamine (TEA) in toluene. The acyl azide (**7a**) was then concerted to the isocyanate via a Curtius Rearrangement. The isocyanate (**8a**) was used immediately after generation and reacted with CMC 2.31 (**9b**) as shown in Scheme 3.9 to yield the title compound.

Scheme 3.9 Synthesis of CMC 2.33



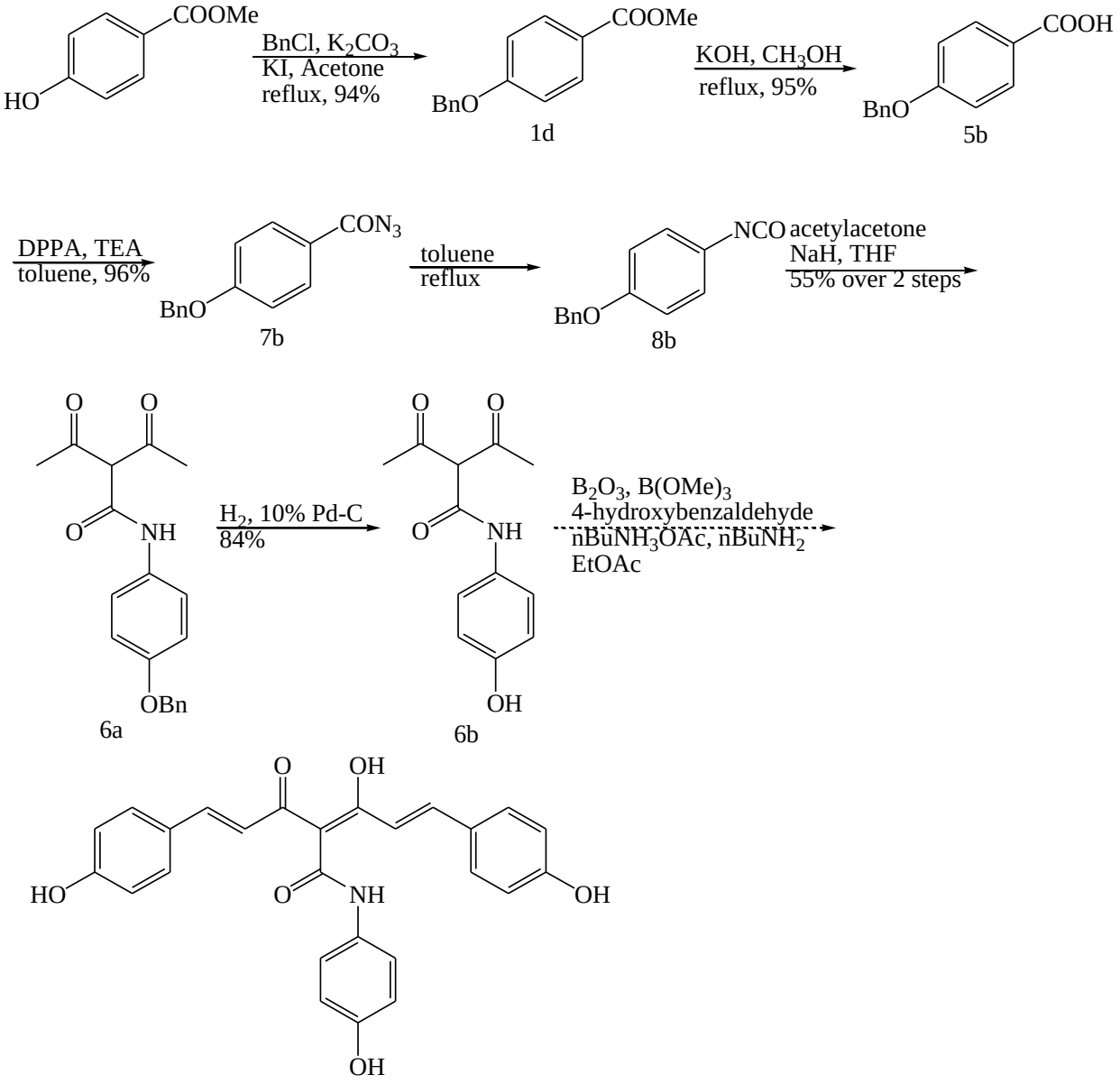
6) Synthesis of CMC 2.34

The 4-substuent on CMC 2.34 (**9e**) is 4-hydroxylphenylaminocarbonyl, and therefore the hydroxyl group must be protected before the isocyanate group can be introduced. This can avoid the reaction between the hydroxyl group and an isocyanate to produce a carbamate molecule [16], which in our case would produce a polymer. Since, tri-carbonyl compounds are not stable in a basic environment, the protective group should not be basic-labile. Thus two groups were chosen, (a) the benzyl group, which can be removed by hydrogenation, and (b) the 2-tetrahydro-2H-pyranoxy (THP) group, which can be cleaved in a mild acid solution.

In order to synthesize CMC 2.34, the benzyl protective group is needed. However, in the deprotection step, the heptadiene backbone could also be hydrogenated, so the deprotection of the benzyl group needs to be performed before the Pabon reaction is carried out. The synthetic scheme is outlined in Scheme 3.10, started with 4-hydroxybenzoic acid methyl ester. The hydroxyl group of the methyl ester was benzylated under basic condition in excellent yield. Following the hydrolysis of the ester gave the corresponding carboxylic acid, was easily converted to the acyl azide in high yield. After refluxing in toluene, the acyl azide was successfully converted to the isocyanate, which was used immediately in the next step to obtain the key intermediate **6a**. This 3-substituted 2,4-pentanedione was hydrogenated to remove the benzyl group, and the hydroxyl product was used in a Pabon-style reaction. Unfortunately, due to the lack of a sufficient amount of **6b** and the difficulty in the experimental Pabon reaction, this method did not give us the desired material. The detailed synthetic approach of this method is illustrated in Scheme 3.10, requiring seven steps.

Scheme 3.10 Synthetic Approach towards CMC 2.34 via the Benzyl Group Protection/Deprotection Method (Method 1)

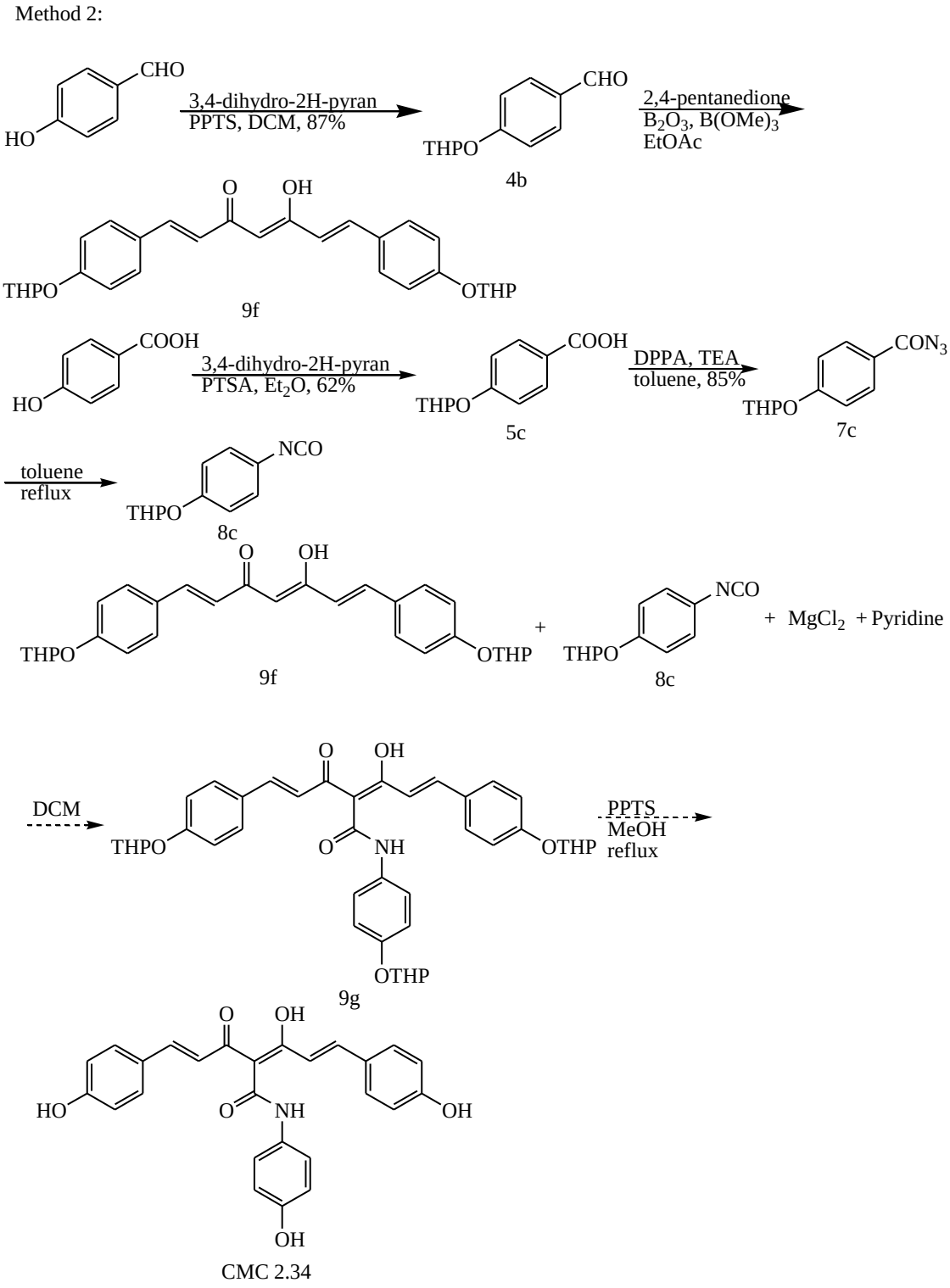
Method 1:



In Method 2, the 2-tetrahydro-2H-pyranoxy (THP) group was chosen to protect the hydroxyl group. This began by protecting the hydroxyl group of both 4-hydroxybenzoic acid and 4-

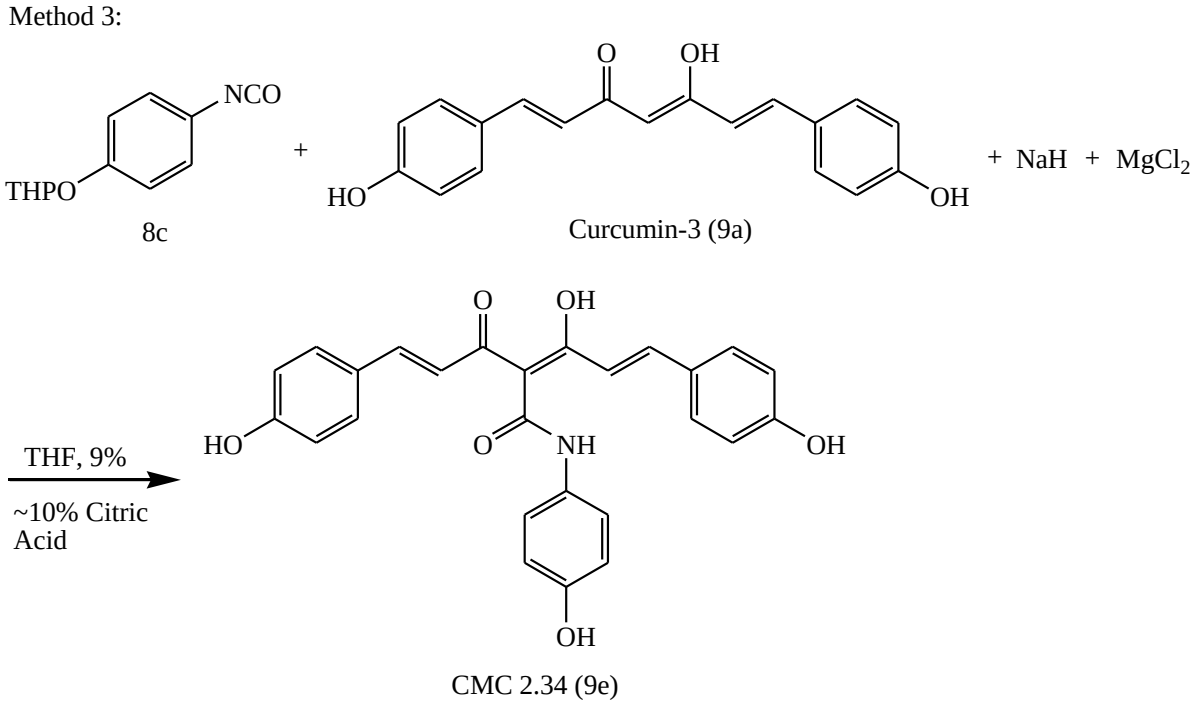
hydroxybenzaldehyde. The THP-protected benzaldehyde was obtained in excellent yield, and was then used in a Pabon reaction to make the bis-THP ether of curcumin-3. The THP-protected benzoic acid then was taken through a series of reactions similar to those noted for the benzyl compound to give the corresponding isocyanate. The isocyanate was then allowed to react with curcumin-3 bis-THP ether in the presence of $MgCl_2$ and pyridine in DCM. ^{1}H-NMR of the crude material showed the presence of a new compound, which was confirmed by the enolic hydrogen peak as well as one phenolic hydrogen peak, and aliphatic hydrogen peaks in the NMR spectrum. The crude product was then refluxed in methanol under the catalysis of PPTS to deprotect the THP group. However, the method was not successful. ^{1}H-NMR showed aliphatic hydrogen peaks, which was an indication of the presence still, of one or more THP groups. This approach was not further pursued since the third method produced the desired material.

Scheme 3.11 Synthetic Approach to CMC 2.34 via the tris-THP Group Protection/Deprotection Variation of Method 2



This method is a variation of the previously described method 2. The 4-O-THP derivative of 4-hydroxyphenyl isocyanate was allowed to react with curcumin-III in the presence of NaH and $MgCl_2$, with an acidic workup using ~10% citric acid aqueous solution, ^{1}H-NMR of the crude material showed very small peaks in the aliphatic hydrogen peak region, which indicated that THP had been almost completely removed by the acid workup. The easy removal of the THP group in the acid workup without a further reaction in acidic media added tremendous simplicity in the experimental work, and this high reactivity is probably due to the nitrogen group para to the THP ring. This para-nitrogen group can serve as a moderate electron donor, which can render the oxygen atom easy to protonate, a necessary step in the deprotection. In the previous work however, the THP protective group was not easy to remove because of the electron-withdrawing group (EWG) para to the THP. The EWG made the oxygen connected to the THP difficult to protonate. Therefore the THP was resistant to easy acid hydrolysis.

Scheme 3.12 Synthesis of CMC 2.34 via the mono-THP Group Protection/Deprotection Variation of Method 3



E. Organic Chemistry Experimental Section

All reagents and solvents used in this experimental work were reagent grade and were used as such, if not otherwise specified. Melting points were taken on a Thomas-Hoover open capillary melting point apparatus and are uncorrected. ^{1}H-NMR spectra were collected on either a Varian Gemini 300 spectrometer or a Bruker Fourier 300 spectrometer in eigher $CDCl_3$ or DMSO-d_6. ^{13}C-NMR spectra were coolected on a Bruker Oxford NMR AS400 spectrometer. Chemical shifts are reported in parts per million (ppm) relative to TMS. IR was taken on Thermo Scientific IR spectrometer with Smart iTR instrument. Mass spectra were recorded on either a Thermo Eletron DSQ GC/MS equipped with a solid probe inlet and EI ionization or an Agilent 1100LC (API-ES)/MSD-VL(m/z=50-1500) using electrospray ionization. Flash chromatography was carried out on a TELEDYNE ISCO CombiFlash Rf machine using 60Å (230-400 mesh) silica

gel (TSI Chemical Co., Cambridge, MA). The starting material and reagents were the best grade commercially available from either Acros, Alfa Aesar or Sigma-Aldrich.

General Procedure for the Synthesis of Substituted Benzoic Acid Methyl Esters

Substituted benzoic acid was dissolved in methanol (1mL per 1mmol of substituted benzoic acid), and 1mL of concentrated sulfuric acid was added. The methanol solution was to reflux overnight. The next day, the methanol solution was poured into water (twice the volume of methanol), the aqueous solution was extracted with DCM. The organic layer was combined and washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4 and evaporate under vacuo to give the desired material.

4-chlorobenzoic acid methyl ester (1a) [16]**:** From 4-chlorobenzoic acid (10.96g, 70mmol), **1a** was isolated as a white solid. Yield: 9.98g, 84% . ^{1}H-NMR ($CDCl_3$, 300MHz): 7.98(d, *J*=8.4Hz, 2H), 7.42(d, *J*=8.4Hz, 2H), 3.92(s, 3H)

2-chlorobenzoic acid methyl ester (1b) [18]: From 2-chlorobenzoic acid (15.67g, 100mmol), **1b** was isolated as a pale orange liquid. Yield: 15.18g, 89%. ^{1}H-NMR ($CDCl_3$, 300MHz): 7.82(dd, *J*=7.8Hz, *J*=1.2Hz, 1H), 7.47~7.38(m, 2H), 7.34~7.28(m,1H), 3.93(s,3H)

2-chloro-4-nitrobenzoic acid methyl ester (1e) [19]: From 2-chloro-4-nitrobenzoic acid (10.08g, 50mmol), **1e** was isolated as a pale yellow solid. Yield: 10.06g, 93%. ^{1}H-NMR ($CDCl_3$, 300MHz): 8.33(d, *J*=2.1Hz, 1H), 8.17(dd, *J*=8.7Hz, *J*=2.1Hz, 1H), 7.98(d, *J*=8.7Hz, 1H), 3.99(s, 3H)

3,5-dinitrobenzoic acid methyl ester (1f): From 3,5-dinitrobenzoic acid (21.22g, 100mmol), **1f** was isolated as a colourless crystal. Yield: 16.15g, 71%. ^{1}H-NMR ($CDCl_3$, 300MHz): 9.24(d,

J=2.1Hz, 1H), 9.18(d, *J*=2.1Hz, 2H), 4.07(s, 3H), ^{13}C-NMR ($CDCl_3$, 300MHz): δ 163.0, 148.6, 133.7, 129.5, 122.4, 53.6. MP: 109~110^{o}C.

3-methyl-4-nitrobenzoic acid methyl ester (1g) [20]**:** From 3-methyl-4-nitrobenzoic acid (9.41g, 50mmol), **1g** was isolated as a pale yellow solid. Yield: 9.64g, 95%. ^{1}H-NMR($CDCl_3$, 300MHz): 8.03(m, 1H), 7.98(m, 2H), 3.96(s, 3H), 2.63(s, 3H)

2-methyl-3-nitrobenzoic acid methyl ester (1h): From 2-methyl-3-nitro-benzoic acid (18.12g, 100mmol), **1h** was isolated as a solid. Yield: 17.30g, 89%. ^{1}H-NMR ($CDCl_3$, 300MHz): 7.99(dd, *J*=8.4Hz, *J*=1.5Hz, 1H), 7.85(dd, *J*=8.4Hz, *J*=1.5Hz, 1H), 7.39(t, *J*=8.4Hz, 1H), 3.96(s, 3H), 2.63(s, 3H), ^{13}C-NMR ($CDCl_3$, 400MHz): δ 166.8, 133.6, 133.4, 133.0, 126.7, 126.4, 52.6, 16.2. MP: 61~62^{o}C.

General Procedure for the Synthesis of Benzylated Acetophenone and Benzoic Acid Methyl Ester

K_2CO_3 (1.1 eq for mono benzylation and 2.2 eq for dibenzylation) was suspended in an acetone solution of 4-hydroxybenzoic acid methyl ester or 4-hydroxyacetophenone or 2,4-dihydroxyacetophenone, then benzyl chloride (1.05 eq for mono benzylation and 2.1 eq for dibenzylation) was added to the suspension followed by catalytic amount of KI. The acetone suspension was allowed to reflux overnight and poured into DCM. The organic layer was washed with water and brine and dried over $MgSO_4$ and the solvent was removed under vacuo to obtain the desired compound.

4-benzyloxybenzoic acid methyl ester (1d) [21]: From 4-hydroxybenzoic acid methyl ester (15.21g, 100mmol), **1d** was isolated as a white solid. Yield: 22.79g, 94%. ^{1}H-NMR ($CDCl_3$,

δ, ppm): 7.96 (d, *J*=9.0Hz, 2H), 7.46-7.32(m, 5H), 7.00(d, *J*=9.0Hz, 2H), 5.12(s, 2H), 3.88(s, 3H)

4-benzyloxyacetophenone (2a) [22]: From 4-hydroxyacetophenone (6.81g, 50mmol), **2a** was isolated as a white solid. Yield: 10.76g, 95%. ^{1}H-NMR ($CDCl_3$, 300MHz): 7.94 (d, *J*=9.0Hz, 2H), 7.46~7.30(m, 5H), 7.01(d, *J*=9.0Hz, 2H), 5.14(s, 2H), 2.56(s, 3H)

2,4-Dibenzyloxyacetophenone (2b) [23]**:** From 2,4-dihydroxyacetophenone (9.13g, 60mmol), **2b** was isolated as a dark pink crystal. Yield: 18.59g, 93%. ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.84(d, *J*=9.0Hz, 1H), 7.46~7.30(m, 10H), 6,62(d, *J*=9.0Hz, 2H), 5.10(d, *J*=7.2Hz, 4H), 2.56(s, 3H)

General Procedure for Methylation of Phenols:

K_2CO_3 (1.2 eq for mono-methylation, 2.4 eq for di-methylation) was suspended in an acetone solution of the substrate, then iodomethane (1.1 eq for mono-methylation, 2.2 equiv for di-methylation) was added to the suspension. The acetone suspension was allowed to reflux overnight and poured into DCM. The organic layer was washed with water and brine and dried over $MgSO_4$ and the solvent was removed under vacuo to obtain the desired compound.

4-methoxybenzoic acid methyl ester (1c) [17]: From 4-hydroxybenzoic acid methyl ester (7.67g, 50mmol), **1c** was isolated as a white solid. Yield: 5.39g, 65%. ^{1}H-NMR ($CDCl_3$, 300MHz): 8.00 (d, *J*=9.0Hz, 2H), 6.92(d, *J*=9.0Hz, 2H), 3.86(s, 3H), 3.62(s, 3H)

3-methoxy-4-nitrobenzoic acid methyl ester (1i): From 3-hydroxy-4-nitrobenzoic acid (1.83g, 10mmol), **1i** was isolated as a yellow solid. Yield: 1.77g, 85%. ^{1}H-NMR ($CDCl_3$, 300MHz): 7.84(d, *J*=8.4Hz, 1H), 7.63(d, *J*=1.5Hz, 1H), 7.67(dd, *J*=8.4Hz, *J*=1.5Hz, 1H), 4.02(s, 3H),

3.97(s, 3H), ^{13}C-NMR ($CDCl_3$, 300MHz): δ 170.2, 165.2, 152.4, 134.8, 125.3, 121.3, 114.5, 56.7, 52.8. MP: 91~92^{o}C.

4-methoxybenzaldehyde (4c) [24]**:** From 4-hydroxybenzaldehyde (4.88g, 40mmol), dark brown liquid. Yield: 5.22g, 96%. ^{1}H-NMR (CDCl3, 300MHz): 9.89(s,1H), 7.85(d, *J*=8.7Hz, 2H), 7.01(d, *J*=8.7Hz, 2H), 3.90(s,3H)

General Procedure for the Synthesis of BAMs

NaH (2.2 equiv, 60% mixed with mineral oil) was placed in a 100mL RBF and washed with hexane. Then 40mL of anhydrous THF was added to the flask and it was cooled down to 0^{o}C. The corresponding acetophenone (2-acetylpyridine for BAM 1.22) and the corresponding benzoic acid methyl ester (ethyl picolinate for BAM 1.22) were added sequentially. The reaction mixture was allowed to warm up to r.t. and stirred overnight. The next day, the reaction was quenched with 10% citric acid and diluted with water. The aqueous layer was extracted with ethyl acetate. The combined organic phase was dried over $MgSO_4$ and evaporated to give a crude material, which was then recrystallized by acetone to yield the desired compound.

1,3-bis-(4-chlorophenyl)-1,3-propanedione (3a) [25]: From **1a** (1.73g, 10mmol) and 4-chloroacetopheone (1.30mL, 10mmol), **3a** was isolated as a pale orange crystal. Yield: 1.60g, 55%. ^{1}H-NMR ($CDCl_3$, 300MHz, 100% enolic): 16.78(s, 1H), 7.92(d, *J*=9.0Hz, 4H), 7.46(d, *J*=9.0Hz, 4H), 6.78(s, 1H)

1,3-bis-(4-benzyloxyphenyl)-1,3-propanedione (3c) [26]: From **1d** (1.13g, 5 mmol) and **2a** (1.21g, 5 mmol), **3c** was isolated as a pale yellow solid. Yield: 1.19g, 55%. ^{1}H-NMR ($CDCl_3$, 300MHz, 100% enolic): 17.12(s, 1H), 7.96 (d, *J*=9.0Hz, 4H), 7.50~7.30(m, 10H), 7.05(d, *J*=9.0Hz, 4H), 5.14(s, 4H)

1-(2-chlorophenyl)-3-(4-chlorophenyl)-1,3-propanedione (3f): From **1b** (1.43mL, 10mmol) and 4-chloroacetophenone (1.30mL, 10mmol), **3f** was isolated as a pale yellow solid. Yield: 1.78g, 61%. ^{1}H-NMR ($CDCl_3$, 300MHz, 100% enolic): 16.30(s, 1H), 7.90(d, *J*=8.7Hz, 2H), 7.68(dd, *J*=8.4Hz, *J*=1.5Hz, 1H), 7.57(d, *J*=8.7Hz, 4H), 7.39(dd, *J*=8.4Hz, *J*=1.5Hz, 1H), 6.71(s, 1H)

1,3-bis-(4-methoxyphenyl)-1,3-propanedione (3g) [27]: From **1c** (3.32g, 20mmol) and 4-methoxyacetophenone (3.00g, 20mmol), **3g** was isolated as a yellow crystal. Yield: 3.12g, 55%. ^{1}H-NMR ($CDCl_3$, 300MHz, 100% enolic): 17.14(s, 1H), 7.96(d, *J*=8.7Hz, 4H), 6.97(d, *J*=8.7Hz, 4H), 6.74(s, 1H), 3.89(s, 6H)

1,3-bis-(2-pyridyl)-1,3-propanedione (3h) [28]: From 2-acetyl pyridine (1.1mL, 10mmol) and ethyl picolinate (1.4mL, 10mmol), **3h** was isolated as a gray solid. Yield: 0.24g, 11%. ^{1}H-NMR ($CDCl_3$, 300MHz, 87% enolic, 13% ketonic): 15.95(s, 1H), 8.75(dq, *J*=4.5Hz, *J*=0.9Hz ,2H), 8.16(d, *J*=9.0Hz, 3H), 7.86(dt, *J*=7.8Hz, *J*=1.5Hz ,2H), 7.42(dq, *J*=4.5Hz, *J*=0.9Hz ,2H). MP: 105~106^{o}C.

General Procedure of Hydrogenation

The substrate was placed in an rbf. 10% Pd-C was added under nitrogen atmosphere (150mg of 10% Pd-C per 1g of substrate). Minimum amount of ethyl acetate of other appropriate solvent was added via syringe. Then nitrogen was removed and the reaction was carried out under hydrogen atmosphere. The reaction progress was monitored by TLC. After completion of the reaction, the reaction solution was filtered through a pad of Celite. The solvent was removed under vacuum to yield the desired compound.

1,3-bis-(4-hydroxyphenyl)-1,3-propanedione (3b) [26, 29]: From **3c** (3.73g, 8.54mmol), **3b** was isolated as a yellow crystal. Yield: 0.82g, 37%. ^{1}H-NMR (DMSO-d_6, 300HMz, 74% enolic, 26% ketonic): 17.59(s, 1H), 10.43(s, 2H), 8.00(d, *J*=8.7Hz, 4H), 7.06(s, 1H), 6.87(d, *J*=9.0Hz, 4H). ESI (-ve) MS m/z 255.0 $[M-H]^-$

1,3-bis-(4-hydroxyphenyl)-2-phenylaminocarbonyl-1,3-propanedione (3e): From **3d** (1.0243g, 1.84mmol), **3e** was isolated as a white solid. Yield: 74.8mg, 11%. ^{1}H-NMR (DMSO-d_6, 300MHz, 100% ketonic): 10.54(s, 2H), 10.29 (s, 1H), 7.78(d, *J*=9.0Hz, 4H), 7.53(d, *J*=8.4Hz, 2H), 7.30(t, *J*=8.4Hz, 2H), 7.05(d, *J*=8.4Hz, 1H), 6.60(d, *J*=9.0Hz, 4H), 6.52(s, 1H). MP: 209~211^{o}C. ESI (-ve) MS m/z 374.0$[M-H]^-$

3-(4-hydroxyphenylaminocarbonyl)-2,4-pentanedione (6b): From **6a** (615mg, 1.86mmol),**6b** was isolated as a pale pink solid. Yield: 373mg, 84%. ^{1}H-NMR(DMSO-d_6, 300HMz, 77% enolic, 23% ketonic): 16.41(s,1H), 10.08(s, 1H), 9.26(s, 1H), 7.41(d, *J*=8.7Hz, 2H), 6.70(d, *J*=8.7Hz, 2H), 2.14(s, 6H), mp 148~150^{o}C. ESI (-ve) MS m/z 234.1 $[M-H]^-$

General Procedure for the Synthesis of Curcumin Analogues

2,4-pentanedione or 3-substituted 2,4-pentanedione (1 eq) and finely powered boron oxide (0.7 eq) were placed in an rbf and heated to 120^{o}C for 5 min. After the pot had been cooled down to r.t., an ethyl acetate (1mL per 1mmol of 2,4-pentanedione or 3-substituted 2,4-pentanedione) solution of trimethyl borate (4 eq) and the corresponding aldehyde (2 eq) was added to the rbf dropwise. Thereafter, with stirring 3-5 drops of 1-butylamine and 3-5 drops of 1-butylammonium acetate in DMF solution (0.136g/mL) was added. The precipitate would appear several hours after the addition of reagents. The reaction mixture was allowed to stir at room temperature for 2 days or longer (1 week is the maximum time). The precipitate was collected by filtration, washed

with diethyl ether and boiled in methanol for half an hour. The methanol was removed by rotary evaporation and the solid crude product was purified by crystallization from methanol and DCM.

1,7-bis-(4-hydroxyphenyl)-1E,6E-heptadien-3,5-dione (9a) [30]: From 2,4-pentanedione (1.2mL, 12mmol) and 4-hydroxybenzaldehyde (2.94g, 24mmol), **9a** was isolated as an orange-red solid. Yield: 2.10g, 57%. ^{1}H-NMR (DMSO-d_6, 300MHz, 100% enolic): 16.37(s, 1H), 10.06(s, 2H), 7.56(d, *J*=8.7Hz, 4H), 7.53(d, *J*=15.9Hz, 2H), 6.81(d, *J*=8.7Hz, 4H), 6.69(d, *J*=15.9Hz, 2H), 6.03(s, 1H). ESI (-ve) MS m/z 307.1 $[M-H]^-$

1,7-bis-(4-acetoxyphenyl)-1E,6E-heptadien-3,5-dione (9b) [31]: From 2,4-pentanedione (1.0mL, 10mmol) and **4a** (3.28g, 20mmol), **9b** was isolated as a yellow crystal. Yield: 1.95g, 49%. ^{1}H-NMR ($CDCl_3$, 300MHz, 100% enolic): 15.87(s, 1H), 7.65(d, *J*=15.6Hz, 2H), 7.58(d, *J*=8.4Hz, 4H), 7.14(d, *J*=8.4Hz, 4H), 6.58(d, *J*=15.6Hz, 2H), 5.84(s, 1H), 2.32(s, 6H). MP: 178~180^{o}C.

1,7-bis-(4-(tetrahydropyran-2-yl)oxyphenyl)-1E,6E-heptadien-3,5-dione (9f): From 2,4-pentanedione (1.0mL, 10mmol) and **4b** (4.12g, 20mmol), **9g** was isolated as an orange solid. Yield: 1.17g, 25%. ^{1}H-NMR ($CDCl_3$, 300MHz, 100% enolic): 16.07(s, 1H), 7.62(d, *J*=15.9Hz, 2H), 7.50(d, *J*=9Hz, 4H), 7.06(d, *J*=9Hz, 4H), 6.50(d, *J*=15.9Hz, 2H), 5.79(s, 1H), 5.48(s, 2H), 3.91~3.84(m, 2H), 3.64~3.60(m, 2H), 2.10~1.86(m, 6H), 1.76~1.56(m, 6H), ^{13}C-NMR ($CDCl_3$, 300MHz): δ 183.3, 158.7, 140.1, 129.6, 128.5, 122.0, 116.7, 96.1, 62.0, 30.2, 25.1, 18.6; ^{13}C-NMR ($CDCl_3$, 400MHz): δ 183.3, 125.7, 140.1, 129.6, 128.5, 122.0, 116.7, 101.4, 96.1, 62.0, 30.1, 25.1, 18.6. MP: 154~155^{o}C. ESI (+ve) MS m/z 477.3 $[M+H]^+$

General Procedure fo the Synthesis of Acyl Azide

The corresponding carboxylic acid was suspended in toluene (1mL per 1mmol of carboxylic acid), and TEA (1.1 eq) and DPPA (1 eq) were added sequentially to the suspension and the suspension became clear solution. The reaction progress was monitored by TLC. After completion of the reaction, the organic layer was washed 3 times with water. The combined aqueous layer was extracted one time with toluene, and the combined organic layer was dried over Na_2SO_4, filtered and the solvent was evaporated to give the title compound.

4-acetoxybenzoyl azide (7a): From **5a** (3.60g, 20mmol), **7a** was isolated as a white solid. Yield: 4.10g, 100%. ^{1}H-NMR ($CDCl_3$, 300MHz): 8.06(d, *J*=9Hz, 2H), 7.20(d, *J*=9Hz, 2H), 2.33(s, 3H). MP: 73~75^{o}C decompose. IR (ATR): 2169, 2133, 1758, 1677, 1599 cm^{-1}

4-benzyloxybenzoyl azide (7b) [32]: From **5b** (5.27g, 23.1mmol), **7b** was isolated as a white solid. Yield: 5.62g, 96% yield. ^{1}H-NMR (CDCl3, 300MHz): 7.98(d, *J*=9Hz, 2H), 7.42~7.36(m, 5H), 7.00(d, J=9Hz, 2H), 5.13(s, 2H). MP: 102~104^{o}C decompose. IR (ATR): 2135, 1680, 1598 cm^{-1}

4-(tetrahydropyran-2-yl)oxybenzoyl azide (7c): From **5c** (3g, 13.5mmol), **7c** was isolated as a pale yellow solid. Yield: 2.85g, 85%. ^{1}H-NMR ($CDCl_3$, 300MHz): 7.97(d, *J*=9Hz, 2H), 7.08(d, *J*=9Hz, 2H), 5.52(t, *J*=3Hz, 1H), 3.88~3.78(m, 1H), 3.68~3.58(m, 1H), 2.08~1.82(m, 3H), 1.76~1.64(m, 3H). MP: 76~78^{o}C decompose. IR (ATR): 2947, 2135, 1679, 1601, 1505 cm^{-1}

General Procedure of the Synthesis of Isocyanate

Acyl azide was placed in a 50mL rbf, toluene (dried over $MgSO_4$, 2mL per 1mmol of acyl azide) was added to dissolve the acyl azide. The toluene solution was heated to reflux for 2h, then

cooled down to r.t. The solvent was removed by rotary evaporation and the remaining residue was used without further purification.

General Procedure of the Reaction between 1,3-dicarbonyl Compounds and Isocyanate

Method 1: NaH (60% mixed with mineral oil) was placed in an rbf and washed with hexane. THF was added to NaH, and the 1,3-dicarbonyl compound was added to the THF suspension of NaH at 0^{o}C, then the isocyanate (neat or in 2mL THF) was added to the THF suspension. After TLC showed complete conversion, the reaction was quenched with 9.6% citric acid and separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layer was washed with brine and dried with Na_2SO_4, filtered and solvent was evaporated. The crude material was recrystallized by proper solvent to yield the desired compound.

Method 2: The 1,3-dicarbonyl compound and $MgCl_2$ (1.15 eq) were suspended in dry DCM and cooled down to -5^{o}C, then pyridine (1.7~2.5 eq) was added to the DCM suspension. After stirring under -5^{o}C for 45 min, the isocyanate (neat or in 2mL of DCM) was added to the DCM suspension. The reaction mixture was then allowed to stir overnight. The next day, the reaction was quenched by 20% citric acid at 0^{o}C and separated. The aqueous layer was extracted with DCM 3 times, and the combined organic layer was washed with 20% citric acid and brine, dried over Na_2SO_4, and the solvent was evaporated. And the crude residue was recrystallized by proper solvent to yield the required material.

Method 3: NaH (60% mixed with mineral oil) was placed in an rbf and washed with hexane. $MgCl_2$ and THF were added to NaH, and the 1,3-dicarbonyl compound was added to the THF suspension at 0^{o}C, then the isocyanate (neat or in 2mL THF) was added to the THF suspension. After TLC showed complete conversion, the reaction was quenched with 9.6% citric acid and

separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layer was washed with brine and dried with Na_2SO_4, filtered and solvent was evaporated to yield the solid residue. The residual solid was then purified by flash chromatography to give the purified compound.

1,3-bis-(4-benzyloxyphenyl)-2-phenylaminocarbonyl-1,3-propanedione (3d): From **3c** (2.94g, 6.75mmol) and phenyl isocyanate (0.74mL, 6.81mmol) by method 1, recrystallized from DMF/isopropanol, **3d** was isolated as a white solid. Yield: 2.61g, 70%. ^{1}H-NMR (DMSO-d_6, 300MHz, 100% ketonic): 10.32(s, 1H), 7.88 (d, *J*=9Hz, 4H), 7.55~7.26(m, 14H), 7.15(d, *J*=9Hz, 4H), 7.06(t, 1H), 6.63(s, 1H), 5.20(s, 4H), ^{13}C-NMR (DMSO-d_6, 400MHz): δ 190.7, 163.7, 162.6, 138.7, 136.3, 130.6, 128.8, 128.8, 128.5, 128.0, 127.8, 123.6, 118.9, 115.0, 69.6, 64.2. MP: 171~173^{o}C. ESI (-ve) MS m/z 554.2 $[M-H]^-$

3-(4-benzyloxyphenylaminocarbonyl)-2,4-pentanedione (6a): From 2,4-pentanedione (1mL, 10mmol) and 4-benzyloxyphenylisocyanate (prepared from **7b**, 2.53g, 10mmol) by method 1, recrystallized from DCM/diethyl ether, **6a** was isolated as a pale yellow solid. Yield: 1.61g, 55% from **7b**. ^{1}H-NMR ($CDCl^3$, 300MHz, 100% enolic): 18.64, 16.39(s, 1H), 11.92(s, 1H), 7.48~7.30(m, 7H), 6.95(d, J=9Hz, 2H), 5.07(s, 2H), 2.50(s, 6H). ESI (-ve) MS m/z 324.1 $[M-H]^-$

3-phenylaminocarbonyl-2,4-pentanedione (6c) [12, 33]: From 2,4-pentanedione (1mL, 10mmol) and phenyl isocyanate (1.1mL, 10mmol) by method 1, recrystallized from DCM/diethyl ether, **6c** was isolated as a white solid. Yield: 1.52g, 69%. ^{1}H-NMR ($CDCl_3$, 300MHz, 100% enolic): 18.54, 16.41(s, 1H), 12.02(s, 1H), 7.51(d, *J*=7.5Hz, 2H), 7.35(d, *J*=7.5Hz, 2H), 7.15(t, *J*=7.15Hz, 1H), 2.50(s, 6H). MP: 117~119^{o}C

1,7-bis-(4-acetoxyphenyl)-4-phenylaminocarbonyl-1E,6E-heptadien-3,5-dione (9c): From **9b** (1.96g, 5mmol) and phenyl isocyanate (0.55mL, 5mmol) by method 2, recrystallized from DCM/diethyl ether, **9c** was isolated as a yellow solid. Yield: 0.63g, 24.5%. ^{1}H-NMR (DMSO-d_6, 300MHz, 100% enolic): 17.33(s,1H), 10.68(s,1H), 7.83(d, *J*=15.6Hz, 2H), 7.73 (d, *J*=8.7Hz, 2H), 7.68(d, *J*=9Hz, 4H), 7.37(t, *J*=7.8Hz, 2H), 7.18(d, *J*=9Hz, 4H), 7.13(m, 1H), 6.90(d, *J*=15.6Hz, 2H), 2.26(s, 6H); ^{13}C-NMR (DMSO-d_6, 400MHz): δ 181.8, 168.9, 164.0, 152.2, 141.5, 138.8, 131.9, 129.7, 128.6, 124.1, 122.6, 120.7, 119.9, 118.1, 115.1, 20.8. MP: 183~185^{o}C. ESI (-ve) MS m/z 510.2 $[M-H]^-$

1,7-bis-(4-acetoxyphenyl)-4-(4-acetoxyphenylaminocarbonyl)-1E,6E-heptadien-3,5-dione (9d): From **9b** (0.48g, 1.22mmol)and **8a** (prepared from **7a**) by method 2, recrystallized from DCM/diethyl ether, **9d** was isolated as a yellow solid. Yield: 0.45g, 65%. ^{1}H-NMR (DMSO-d_6, 300MHz, 100% enolic): 17.36(s, 1H), 10.74(s, 1H), 7.83(d, *J*=15.6Hz, 2H), 7.74(d, *J*=9Hz, 2H), 7.70(d, *J*=8.4Hz, 4H), 7.18(d, *J*=8.4Hz, 4H), 7.13(d, *J*=9Hz, 2H), 6.91(d, *J*=15.6Hz, 2H), 2.26(s, 9H); ^{13}C-NMR (DMSO-d_6, 400MHz): δ 181.8, 169.4, 168.9, 152.2, 141.6, 131.9, 129.8, 129.6, 124.3, 122.6, 122.4, 122.1, 120.9, 120.7, 20.8, 20.8. MP: 176~177^{o}C. ESI (-ve) MS m/z 568.2 $[M-H]^-$

1,7-bis-(4-hydroxyphenyl)-4-(4-hydroxylphenylaminocarbonyl)-1E,6E-heptadien-3,5-dione (9e): From **9a** (1.41g, 3.7mmol) and **8c** (prepared from **7c**) by method 3, chromatographed from acetone:heptane 1:2 ~ 3:2, 9e was isolated as a red solid. Yield: 142mg, 9%. ^{1}H-NMR (DMSO-d_6, 300MHz, 100% enolic): 17.49(s, 1H), 10.35(s, 1H), 10.15(s, 2H), 9.32(s, 1H), 7.69(d, *J*=15.6Hz, 2H), 7.50(d, *J*=9Hz, 2H), 7.45(d, *J*=8.9Hz, 4H), 6.80(d, *J*=8.9Hz, 4H), 6.76(d, *J*=9Hz, 2H), 6.67(d, *J*=15.6Hz, 2H); ^{13}C-NMR (DMSO-d_6, 400MHz): δ 182.0, 164.4, 160.7, 154.4,

142.7, 131.1, 130.9, 125.9, 122.0, 117.6, 116.6, 115.7. MP: decomposed when heated above 120^{o}C. ESI (-ve) MS m/z 442.1 $[M-H]^-$

Others:

4-acetoxybenzaldehyde (4a) [34]: 4-hydroxybenzaldehyde (6.1g, 50mmol)was suspended in DCM (50mL), then TEA (7.7mL, 55mmol) and acetyl chloride (3.9mL, 55mmol) were added to the suspension sequentially. After TLC showed complete conversion, the organic layer was washed with water, saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and the solvent was evaporated in vacuum to give the title compound as a brownish liquid. Yield: 7.88g, 96%. ^{1}H-NMR ($CDCl_3$, 300MHz): 10.04(s, 1H), 7.97(d, *J*=9Hz, 2H), 7.33(d, *J*=9Hz, 2H), 2.39(s, 3H)

4-(tetrahydropyran-2-yl)oxybenzaldehyde (4b) [35]: 4-hydroxylbenzaldehyde (5.01g, 41mmol) was suspended with PPTS(0.21g, catalyst) in DCM, and 3,4-dihydro-2H-pyran (10.4mL, 114mmol) was added to the suspension. After TLC showed complete conversion, the organic layer was washed with brine twice, and dried over Na_2SO_4, the solvent was then evaporated to yield a sticky oily residue. The oily material was purified by flash chromatography to give a pale yellow oil. Yield: 7.36g, 87%. ^{1}H-NMR ($CDCl_3$, 300MHz): 9.88(s, 1H), 7.82 (d, *J*=9Hz, 2H), 7.14(d, *J*=9Hz, 2H), 5.53(t, *J*=3Hz, 1H), 3.88~3.78(m, 1H), 3.68~3.56(m, 1H), 2.08~1.82(m, 3H), 1.80~1.64(m, 3H)

4-acetoxybenzoic acid (5a) [36]: 4-hydroxylbenzoic acid (20.73g, 150mmol) was stirred in acetic anhydride (50mL), and pyridine (50mL) was added to the suspension. The mixture was allowed to stir overnight. The next day the mixture was poured into 400mL of H_2O, and stirring continued for 30 min, white crystal appeared. The pH of the aqueous suspension was adjusted to ~ 2 by using concentrated HCl and the white crystal was filtered and washed with H_2O and dried

to yield the title compound as a colourless/white crystal. Yield: 23.51g, 87%. ^{1}H-NMR ($CDCl_3$, 300MHz): 8.15(d, *J*=9Hz, 2H), 7.22(d, *J*=9Hz, 2H), 2.34(s, 3H)

4-benzoyloxybenzoic acid (5b) [37]: 4-benzyloxybenzoic acid methyl ester (**1d**) (9.69g, 40mmol) and NaOH (2.4g, 60mmol) were refluxed in methanol overnight. After the reaction pot cooled down to r.t., the white precipitate was filtered and collected, and then poured into 20% H_2SO_4. After stirring for 30 min, the white precipitate was filtered and washed and dried to give the title compound as a white solid. Yield: 8.68g, 95%. ^{1}H-NMR (DMSO-d_6, 300MHz): 7.88(d, *J*=9Hz, 2H), 7.45~7.35(m, 5H), 7.08(d, *J*=9Hz, 2H), 5.18(s, 2H)

4-(tetrahydropyran-2-yl)oxybenzoic acid (5c) [38]: 4-hydroxybenzoic acid (2.76g, 20mmol) and PTSA (0.17g, catalyst) were suspended in diethyl ether (20mL). 3,4-dihydro-2H-pyran (2.7mL, 30mmol) was then added to the reaction mixture dropwise. The reaction mixture turned pink immediately, then precipitate started to form. After stirring overnight, the reaction mixture was filtered and the precipitate was washed with diethyl ether and dried to yield the product as a white powder. Yield: 2.76g, 62%. ^{1}H-NMR ($CDCl_3$, 300MHz): 8.05(d, *J*=9Hz, 2H), 7.10(d, *J*=9Hz, 2H), 5.53(t, *J*=3Hz, 1H), 3.92~3.78(m, 1H), 3.68~3.56(m, 1H), 2.08~1.82(m, 3H), 1.80~1.64(m, 3H)

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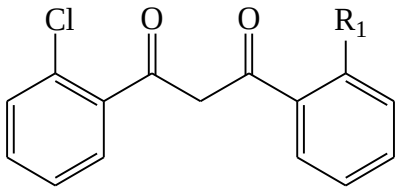
Chapter 4 Conclusion and Future Work

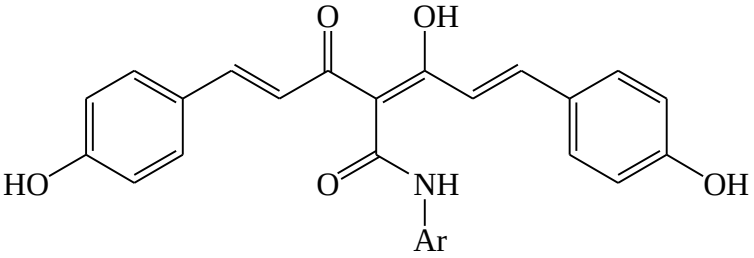
To conclude, in this thesis, 8 different BAMs were synthesized by using the corresponding benzoic acid methyl ester derivative and the corresponding substituted acetopheone via a Claisen-type condensation reaction. 7 of the synthesized BAMs were tested. The most potent one is BAM 1.20 [1-(2-chlorophenyl)-3-(4-chlorophenyl)-1,3-propanedione], with an IC_{50} of 5.2±0.1μM against MMP-9 in vitro. In the CMC part, 5 CMCs and a natural product curcumin-3 were synthesized successfully, and the synthesis uttilized a combined strategy of protective group chemistry, Pabon's reaction and reaction of 1,3-dicarbonyl compounds with isocyanates.

Though the CMC family has grown to the size of 34 members, it is still an underdeveloped field with great research potential. CMC 2.24 and the newly synthesized derivatives all share the 4-substituted-phenylaminocarbonyl group, and there are still a lot of variations to explore Analogues with 2-substituted-phenyl or 3-substituted-phenyl ring have never been synthesized. Other analogues which possess heterocyclic ring instead of a phenyl ring have not been reported. In the BAM series, however, because the 2-chloro substituent on one phenyl ring can greatly change the inhibitory effect, therefore it is worthwhile to produce other analogues with 2-substituent (Figure 4.1). The biological work in this thesis is only preliminary, the inhibitors need to be tested against other MMPs to get an overview, and also necessary to guide the research into the next stage.

Figure 4.1 Suggested Future Synthetic Targets



3i: R_1=H
3j: R_1=Cl



9g: Ar=2-hydroxyphenyl
9h: Ar=3-hydroxyphenyl
9i: Ar=4-dimethylaminophenyl
9j: Ar=2-methylphenyl
9k: Ar=2-furyl

Appendix 1 Compound Characterization Checklist

Table 5.1 Compound Characterization Checklist

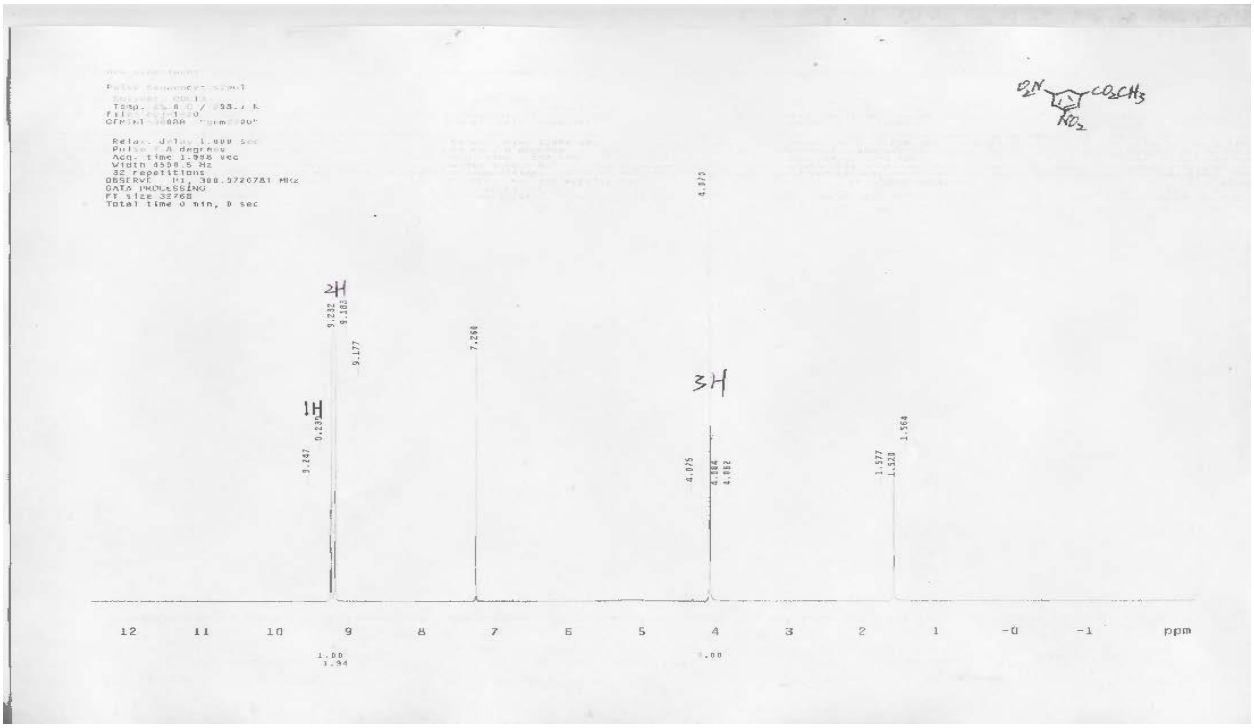
Compound table-entry number	New	Known	Weight and percentage yield	Physical state / mp range if cryst. Solid	IR	^{1}H-NMR	^{13}C-NMR	MS
1a		**X**	**X**	**X**		**X**		
1b		**X**	**X**	**X**		**X**		
1c		**X**	**X**	**X**		**X**		
1d		**X**	**X**	**X**		**X**		
1e		**X**	**X**	**X**		**X**		
1f	**X**		**X**	**X**		**X**	**X**	
1g		**X**	**X**	**X**		**X**		
1h	**X**		**X**	**X**		**X**	**X**	
1i	**X**		**X**	**X**		**X**	**X**	
2a		**X**	**X**	**X**		**X**		
2b		**X**	**X**	**X**		**X**		
3a		**X**	**X**	**X**		**X**		

Compound table-entry number	New	Known	Weight and percentage yield	Physical state / mp range if cryst. Solid	IR	^{1}H-NMR	^{13}C-NMR	MS
3b		**X**	**X**	**X**		**X**		**X**
3c		**X**	**X**	**X**		**X**		
3d	**X**		**X**	**X**		**X**	**X**	**X**
3e	**X**		**X**	**X**		**X**		**X**
3f		**X**	**X**	**X**		**X**		
3g		**X**	**X**	**X**		**X**		
3h		**X**	**X**	**X**		**X**		
4a		**X**	**X**	**X**		**X**		
4b		**X**	**X**	**X**		**X**		
4c		**X**	**X**	**X**		**X**		
5a		**X**	**X**	**X**		**X**		
5b		**X**	**X**	**X**		**X**		
5c		**X**	**X**	**X**		**X**		

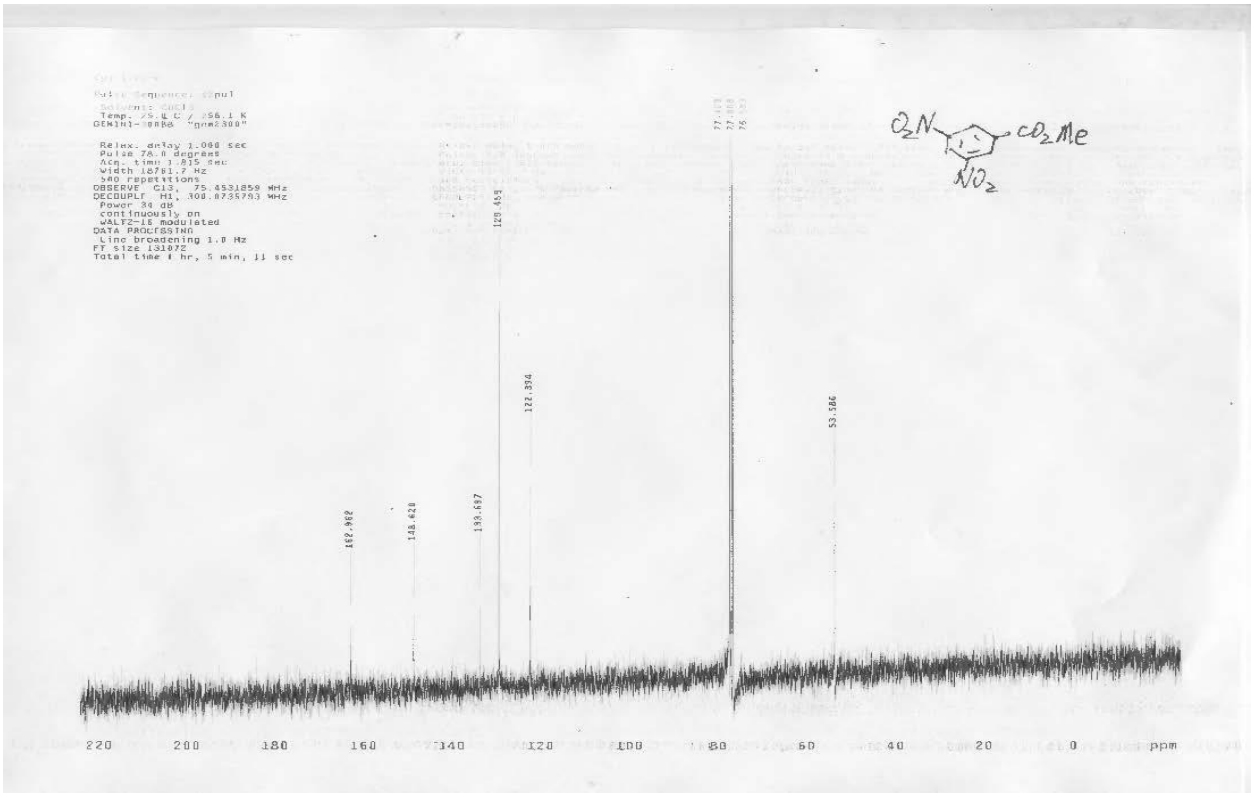
Compound table-entry number	New	Known	Weight and percentage yield	Physical state / mp range if cryst. Solid	IR	^{1}H-NMR	^{13}C-NMR	MS
6a	X		X	X		X		X
6b	X		X	X		X		X
6c		X	X	X		X		
7a		X	X	X	X	X		
7b		X	X	X	X	X		
7c	X		X	X	X	X		
9a		X	X	X		X		X
9b		X	X	X		X		
9c	X		X	X		X	X	X
9d	X		X	X		X	X	X
9e	X		X	X		X	X	X
9f	X		X	X		X	X	X

Appendix 2 ^{1}H-NMR and ^{13}C-NMR Spectra of Some Synthesized Compounds

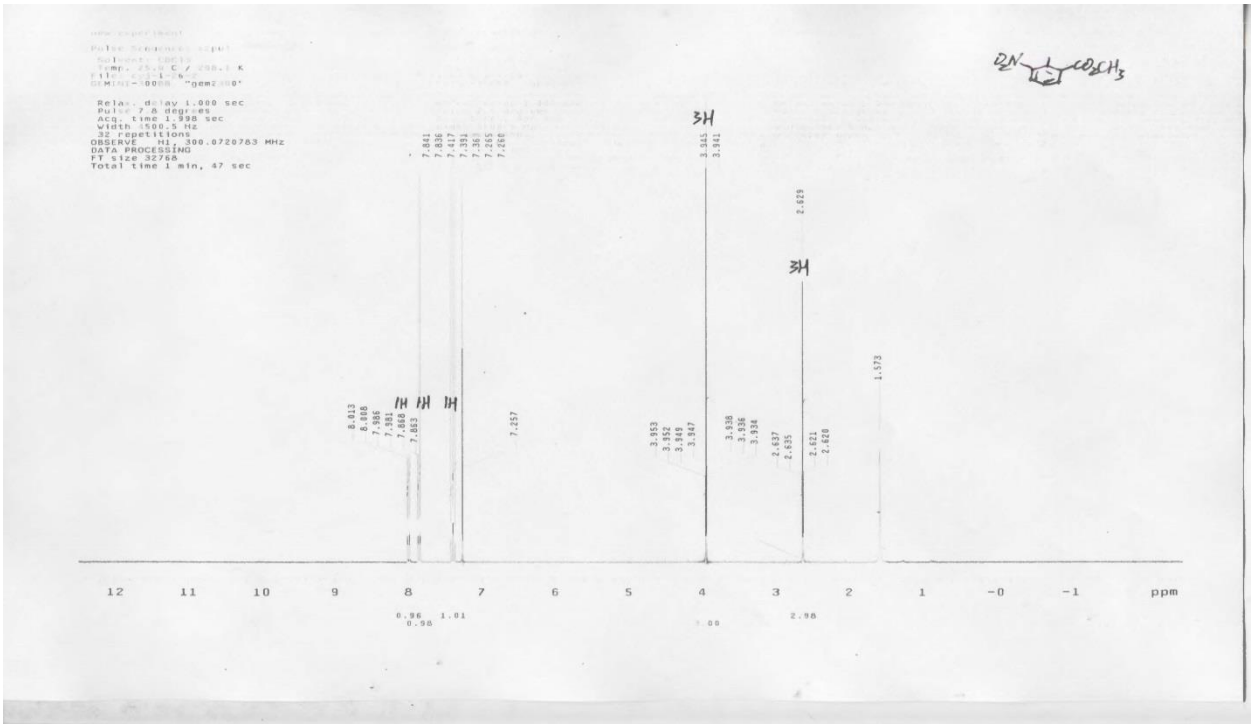
List of ^{1}H-NMR and ^{13}C-NMR Spectra



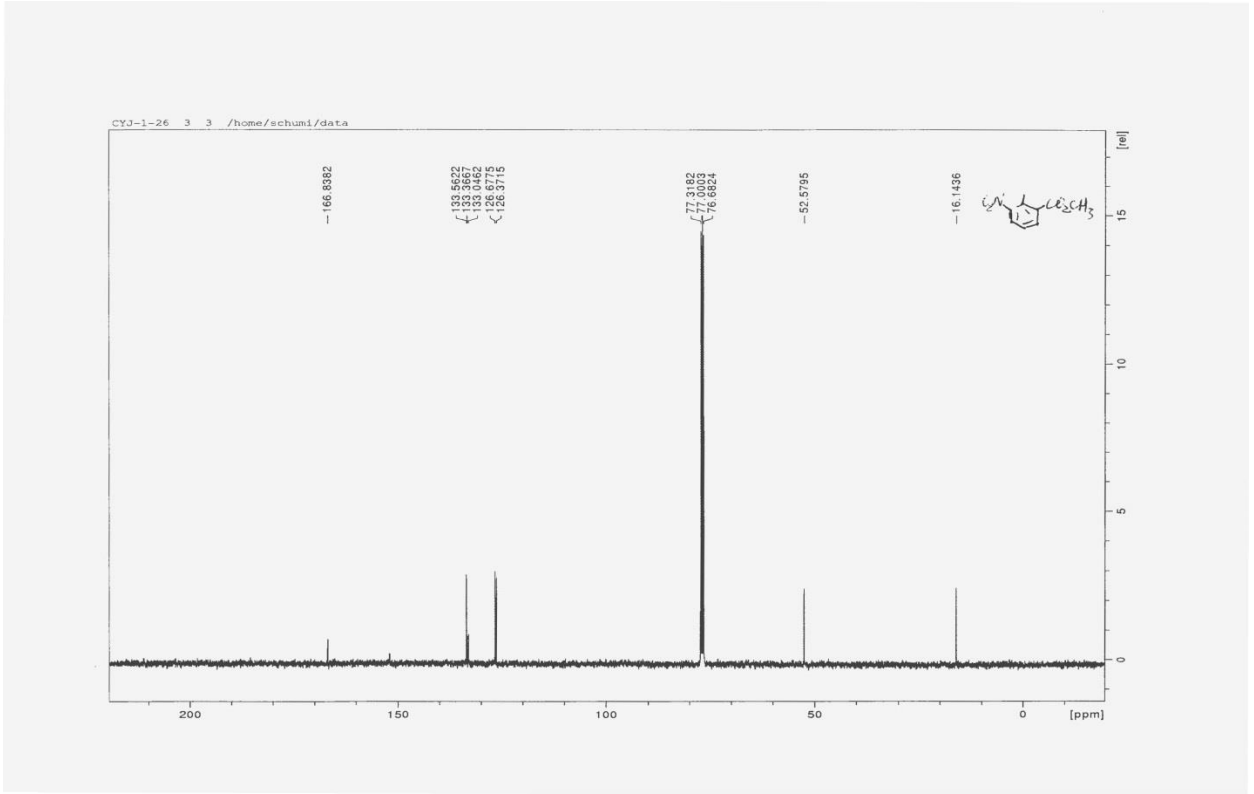
^{1}H-NMR Spectrum of 1f



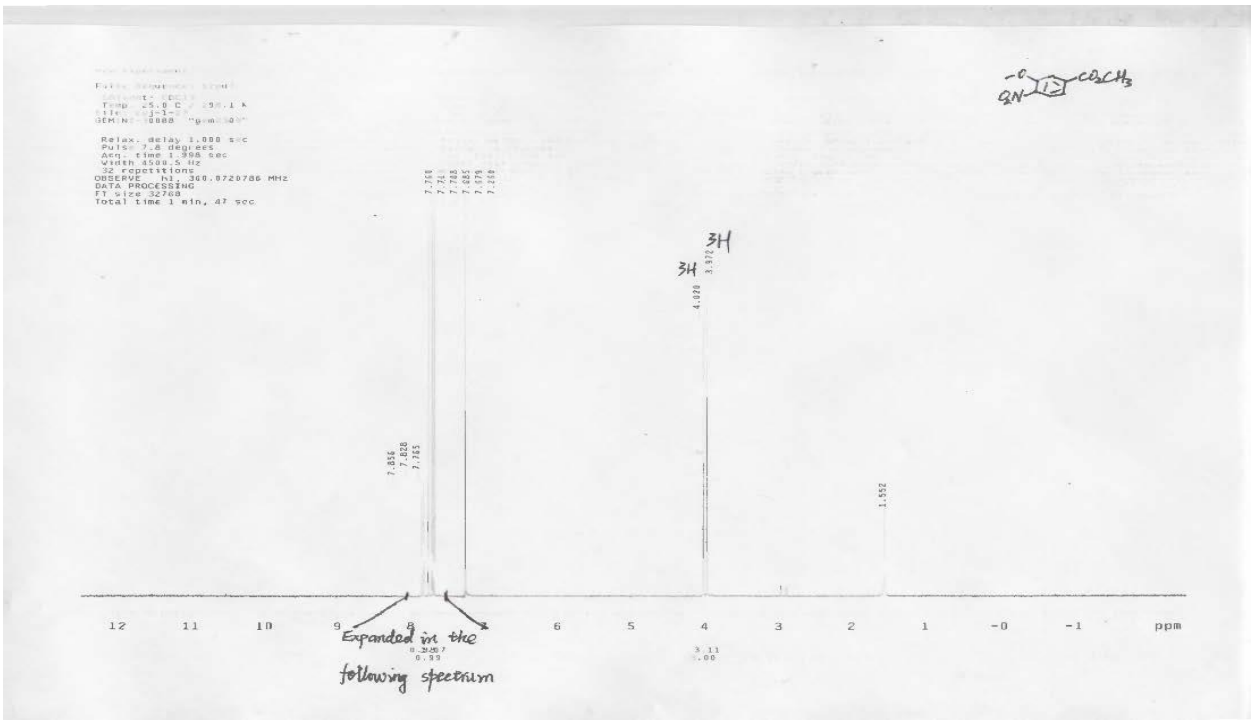
^{13}C-NMR Spectrum of 1f



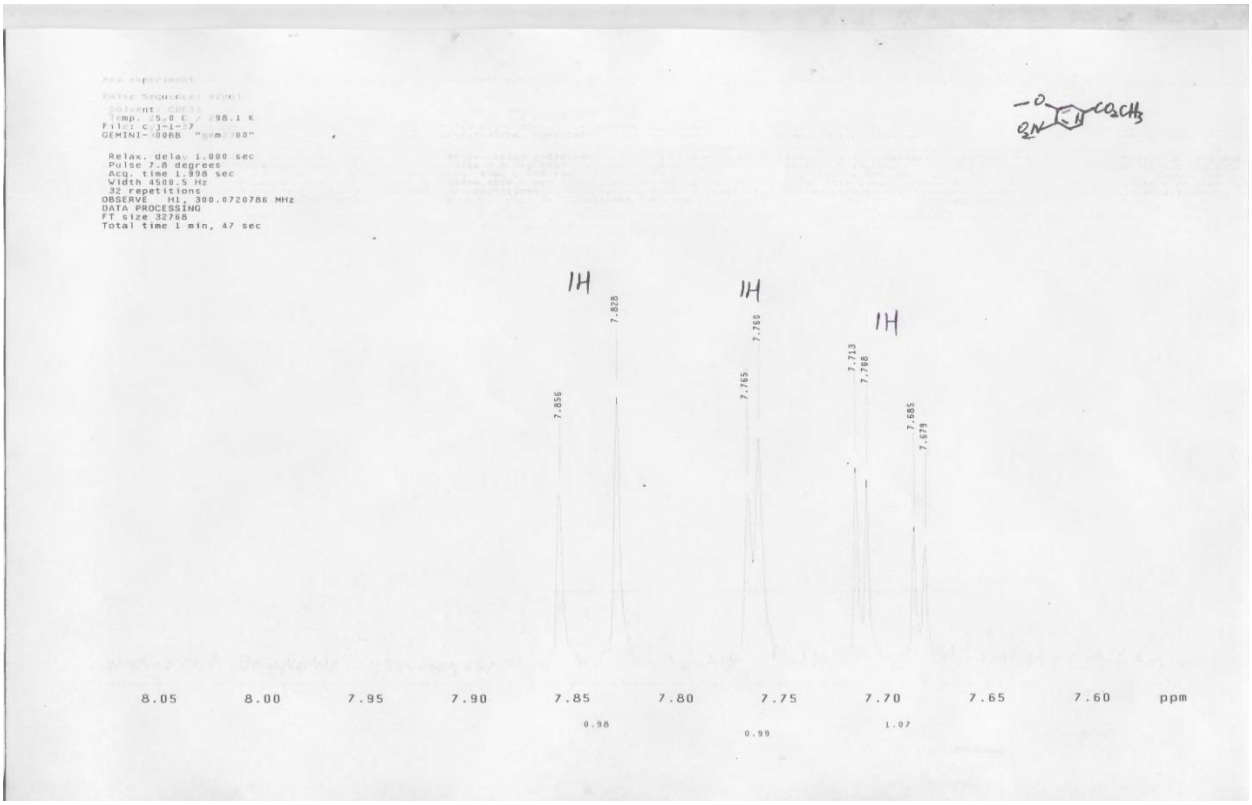
^{1}H-NMR Spectrum of 1h



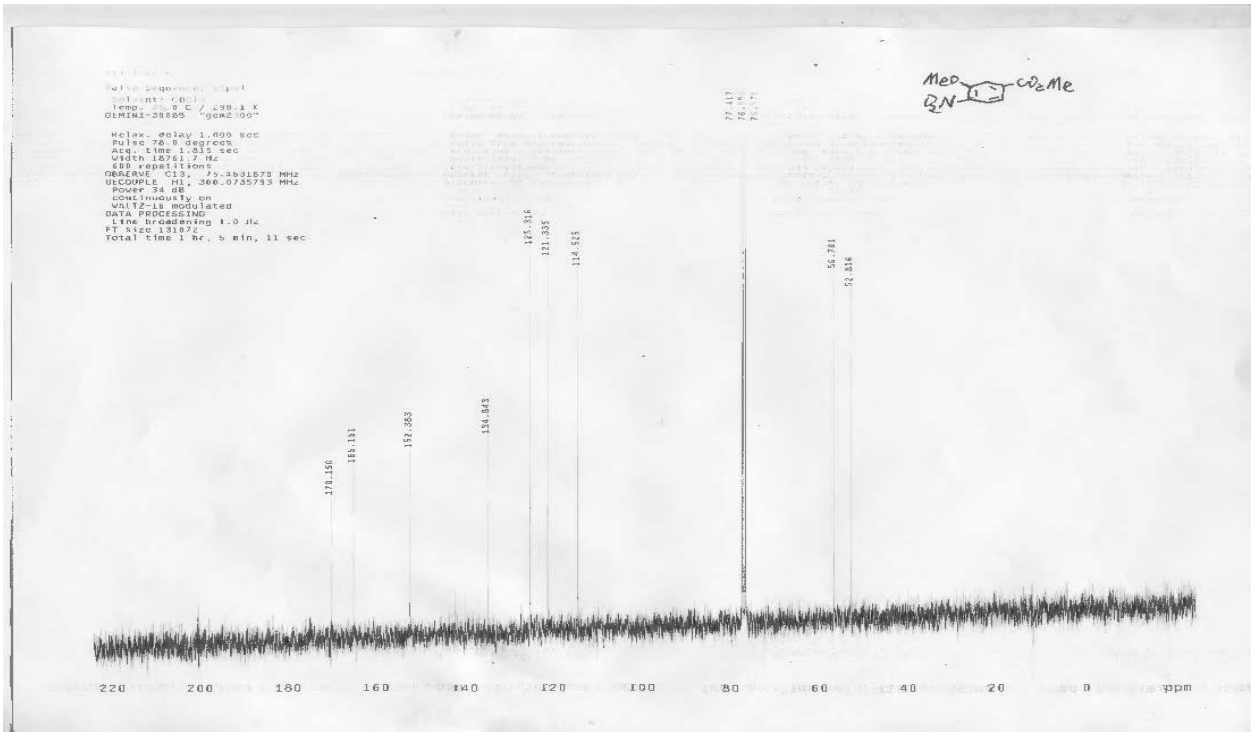
^{13}C-NMR Spectrum of 1h



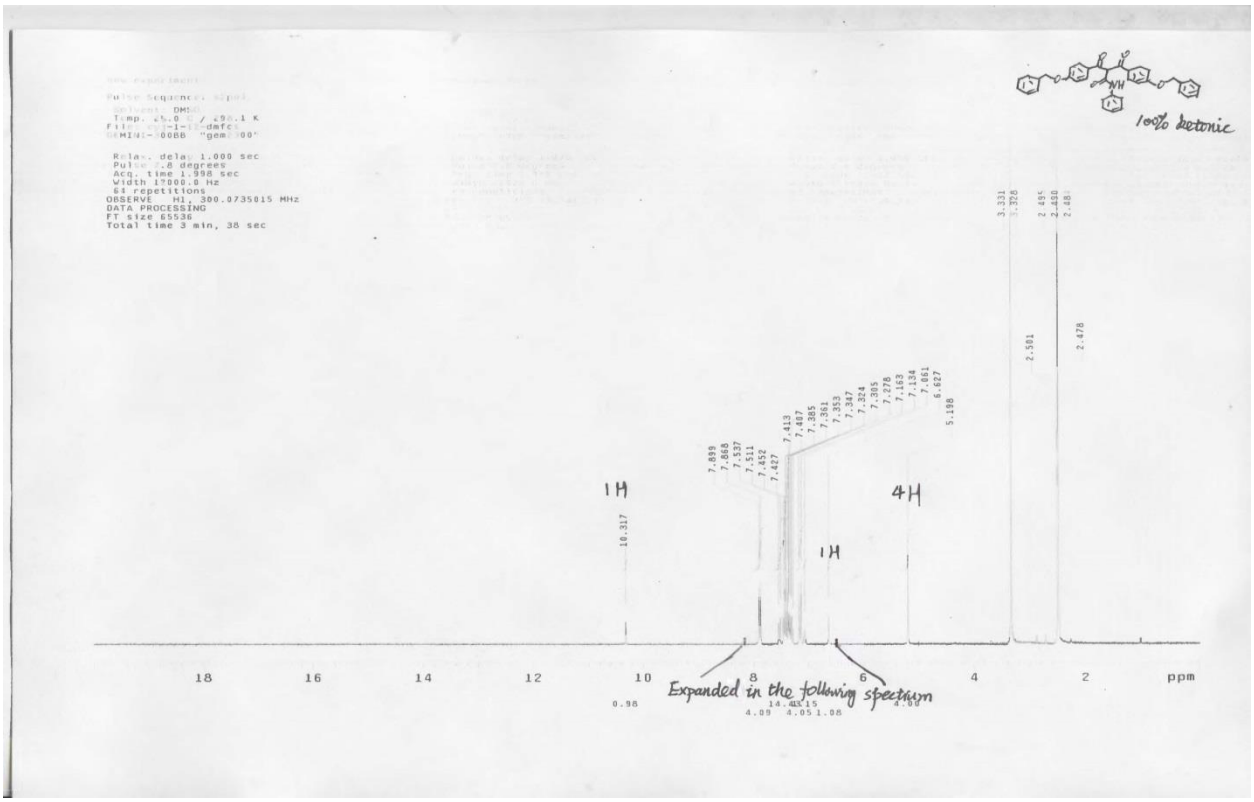
^{1}H-NMR Spectrum of 1i



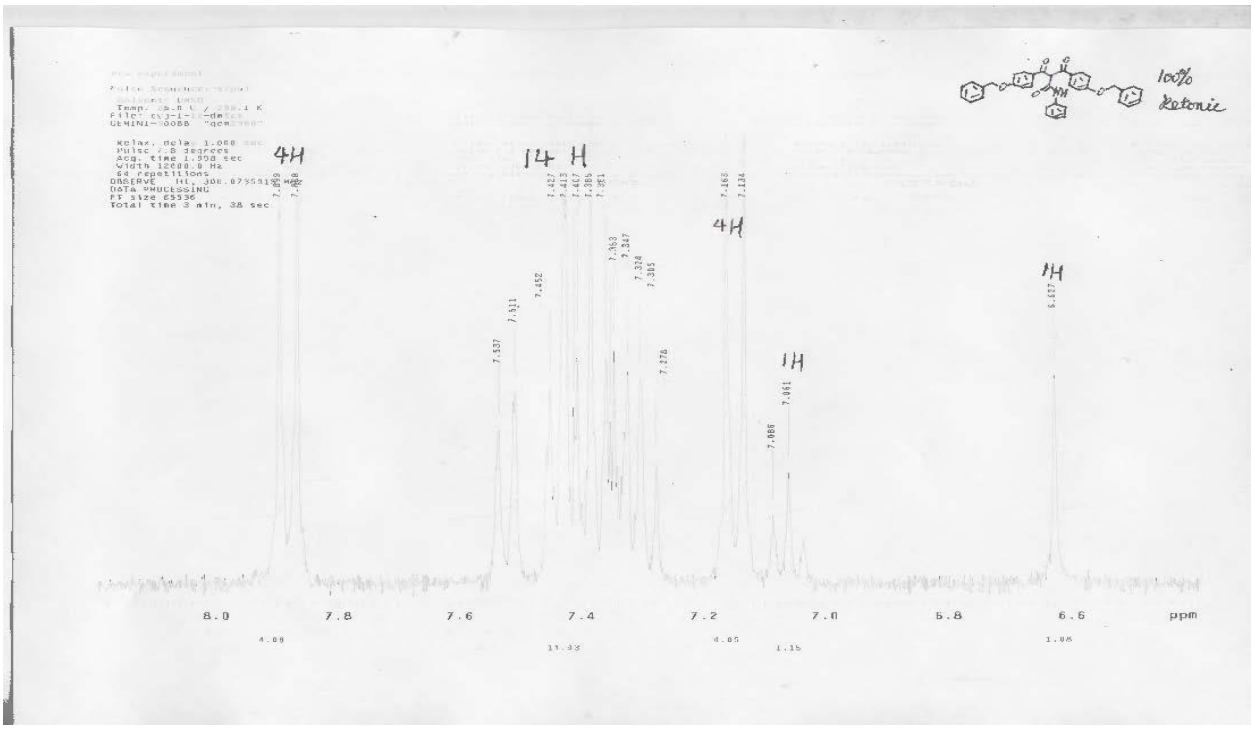
^{1}H-NMR Spectrum (Expanded from δ 8.10~7.55) of 1i



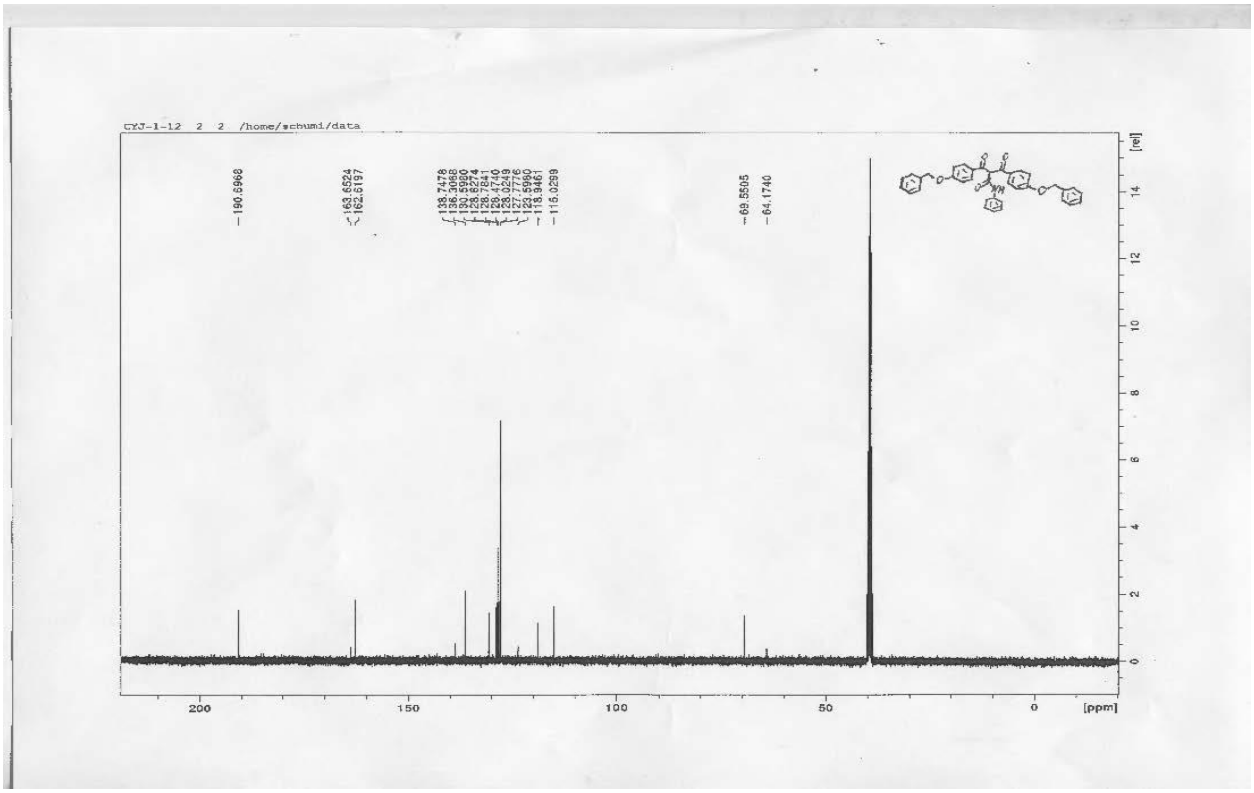
^{13}C-NMR Spectrum of 1i



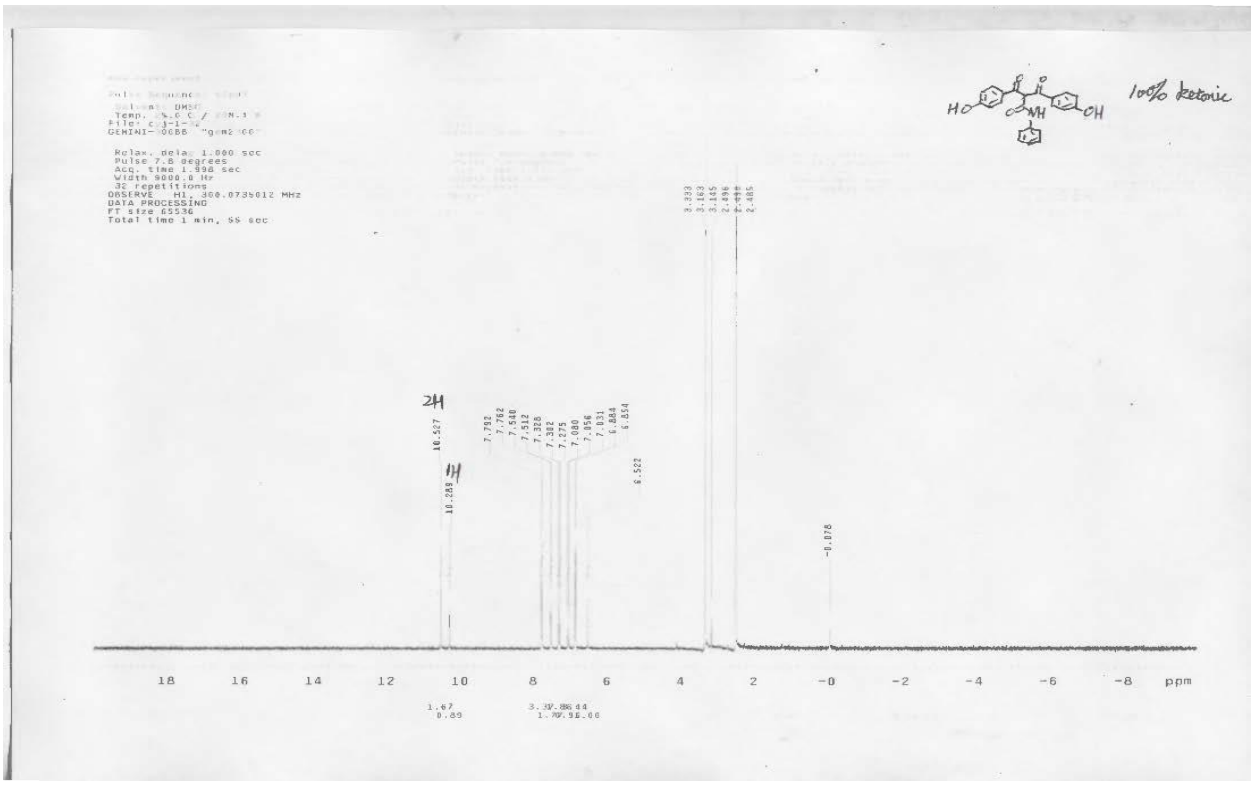
^{1}H-NMR Spectrum of 3d



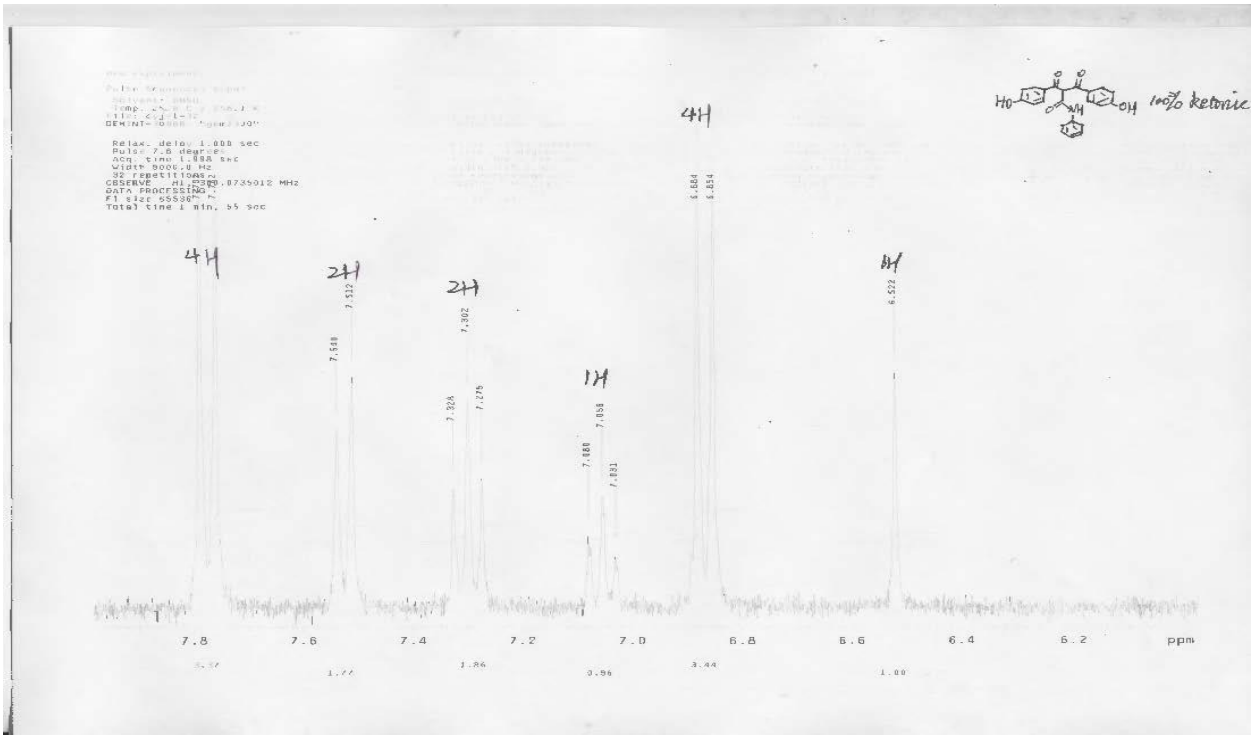
[1]H-NMR Spectrum (Expanded from δ 8.10~6.40) of 3d



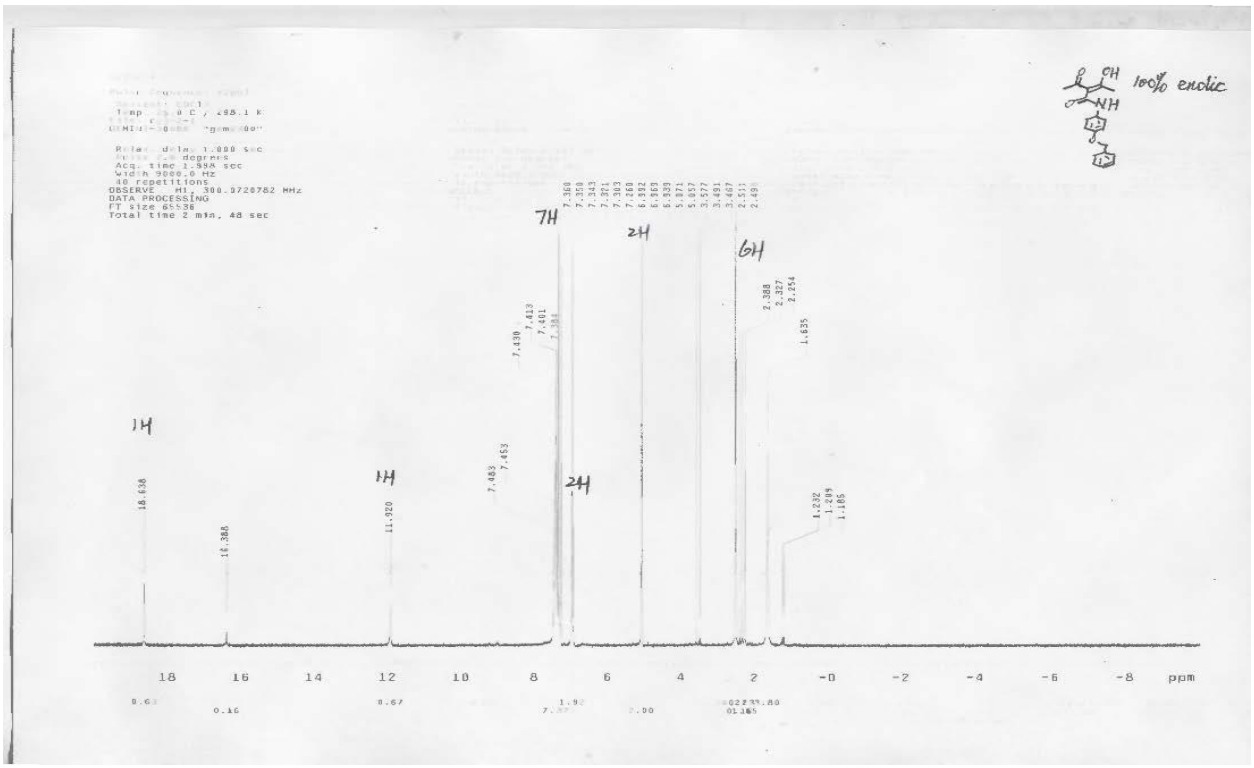
[13]C-NMR Spectrum of 3d



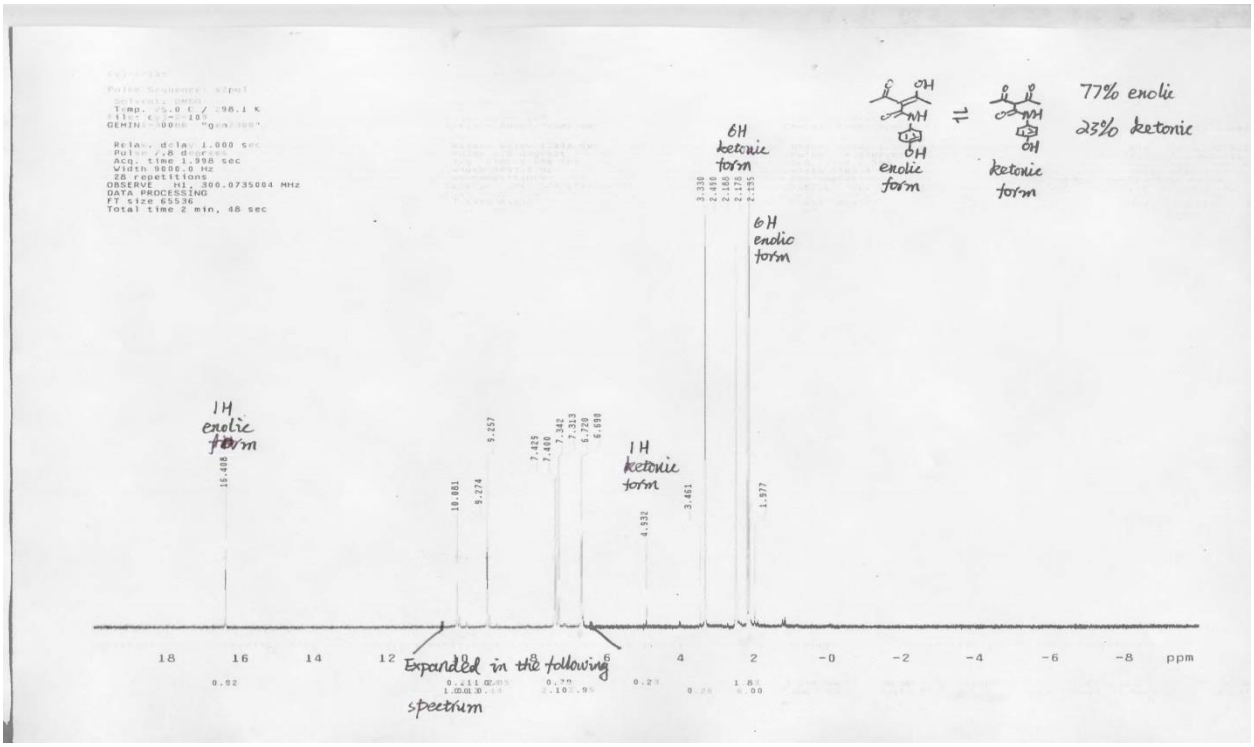
^{1}H-NMR Spectrum of 3e



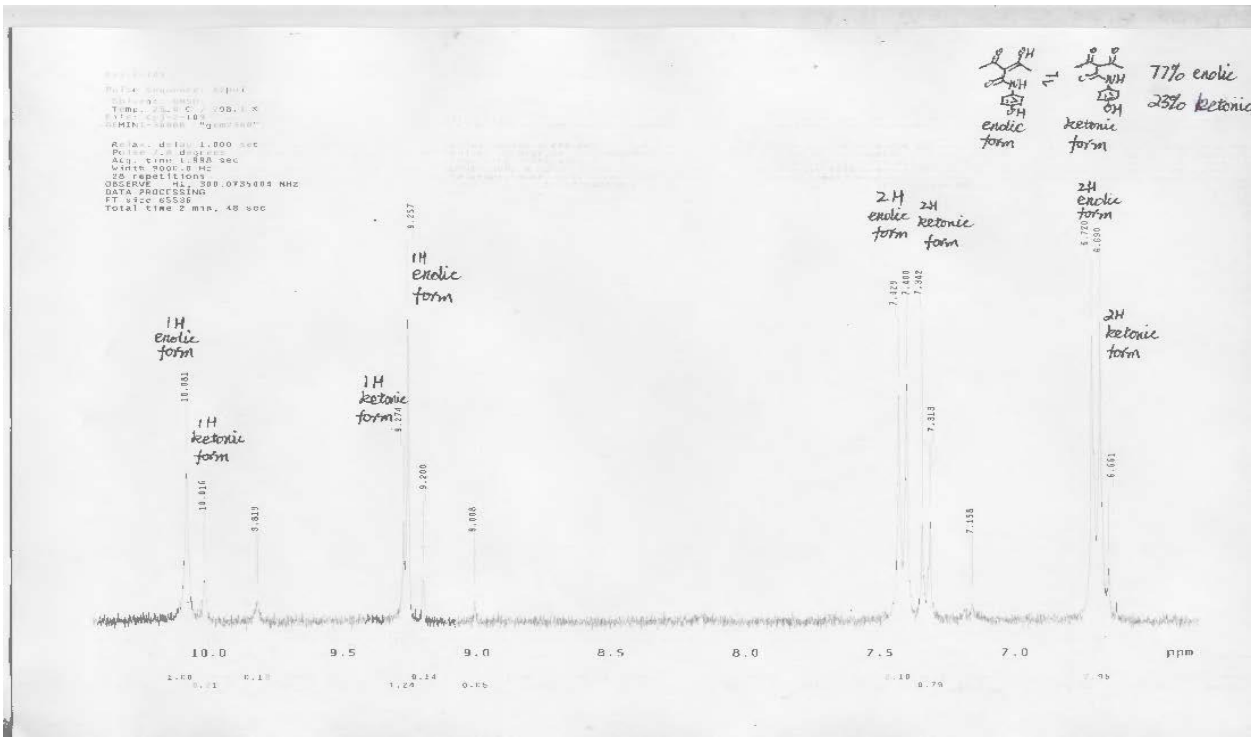
^{1}H-NMR Spectrum (Expanded from δ 7.90~6.50) of 3e



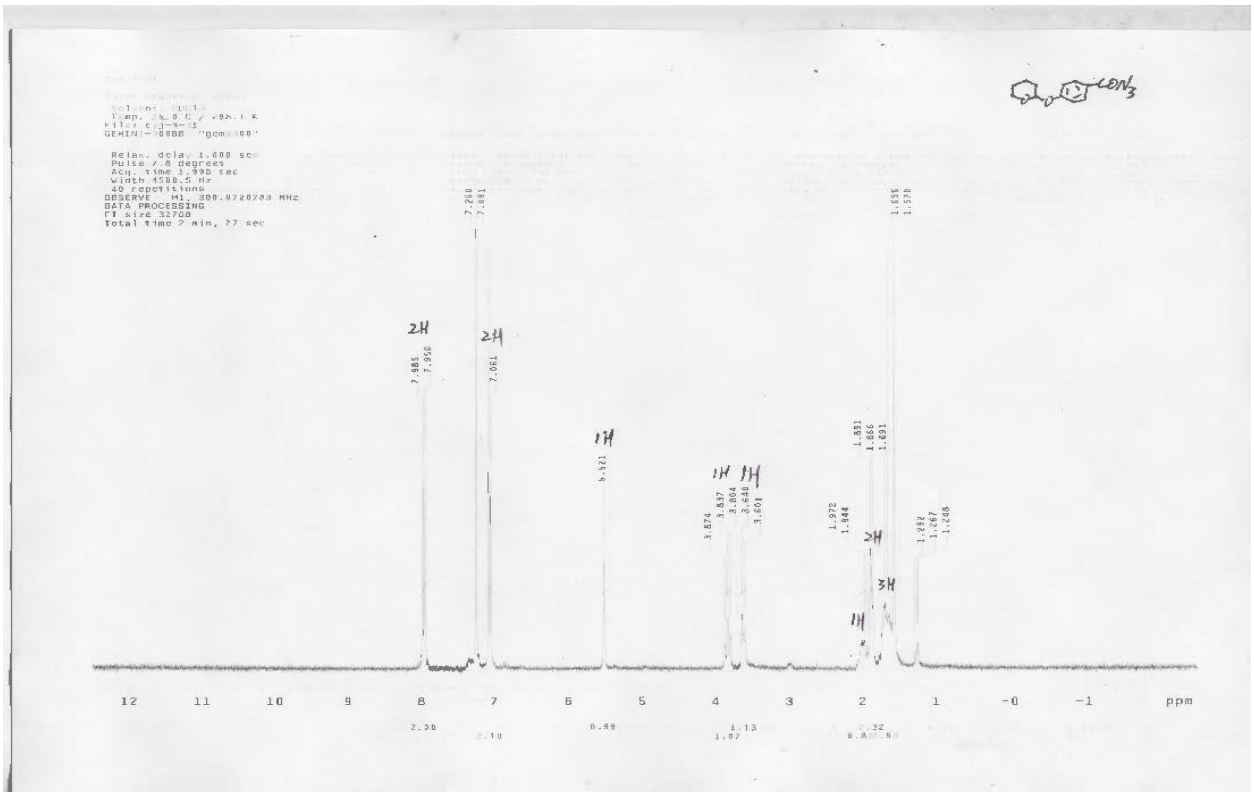
^{1}H-NMR Spectrum of 6a



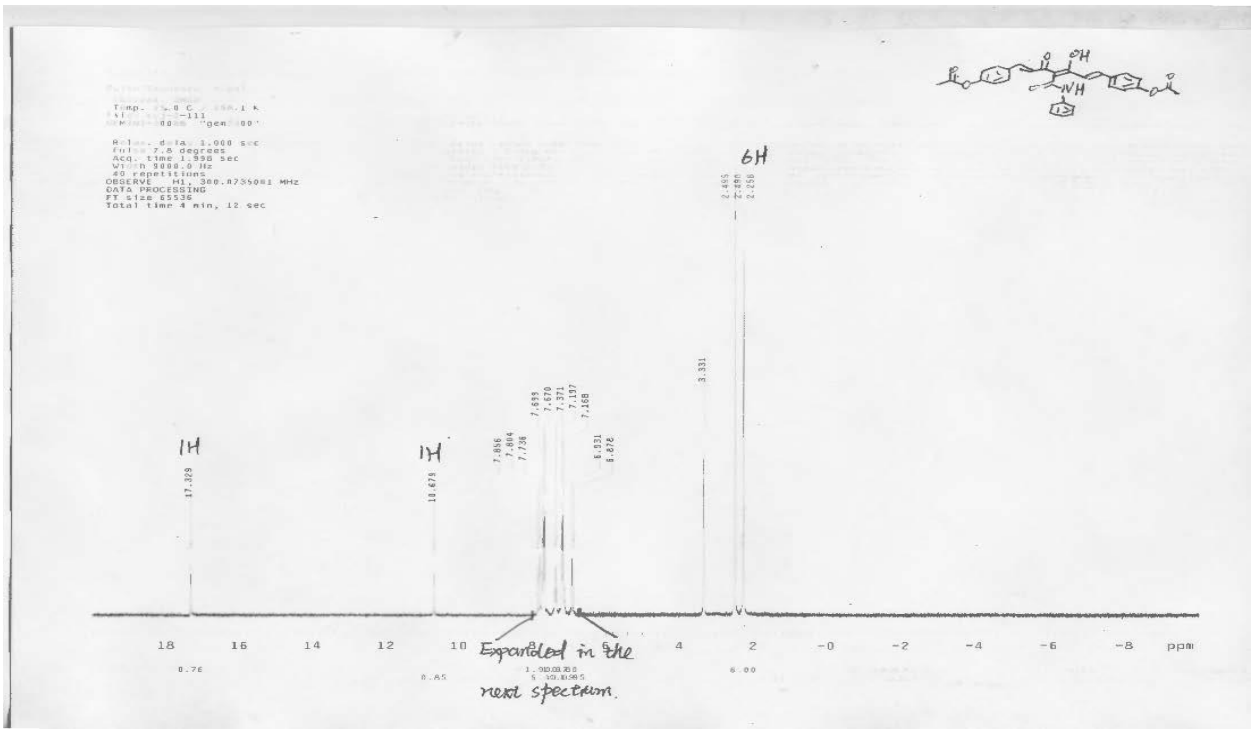
^{1}H-NMR Spectrum of 6b



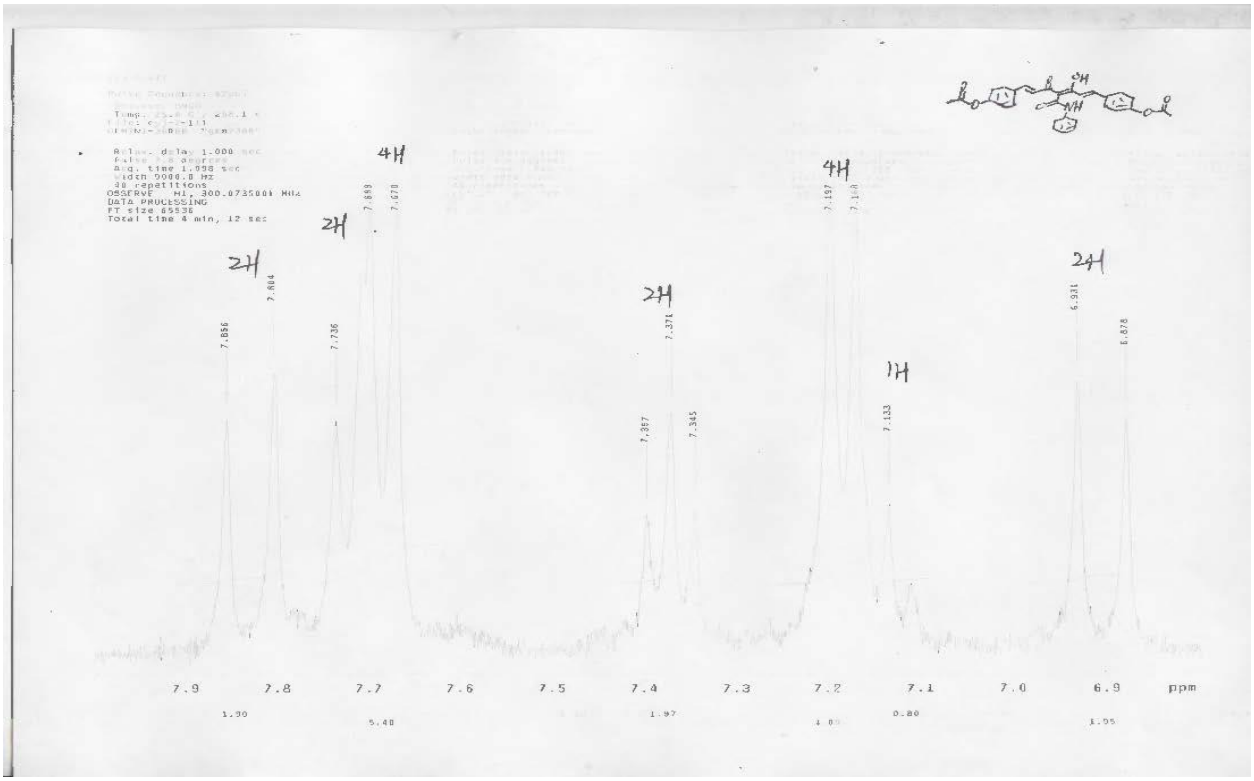
[1]H-NMR Spectrum (Expanded from δ 10.20~6.45) of 6b



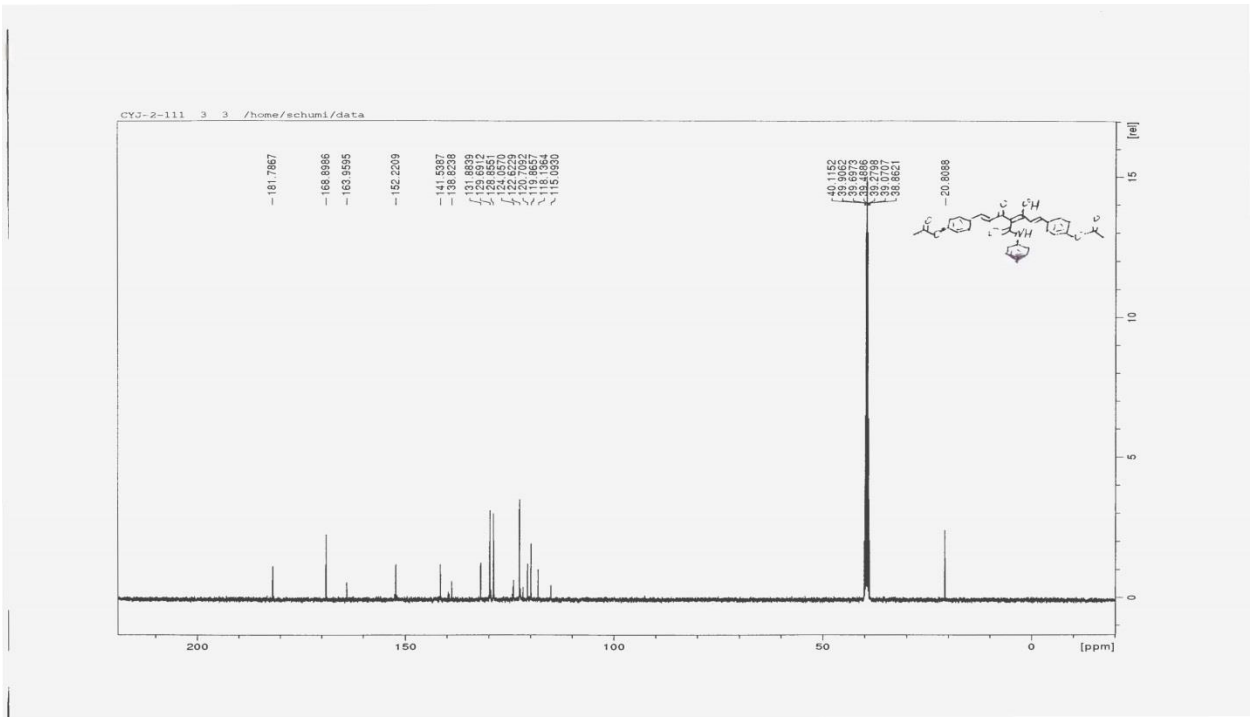
[1]H-NMR Spectrum of 7c



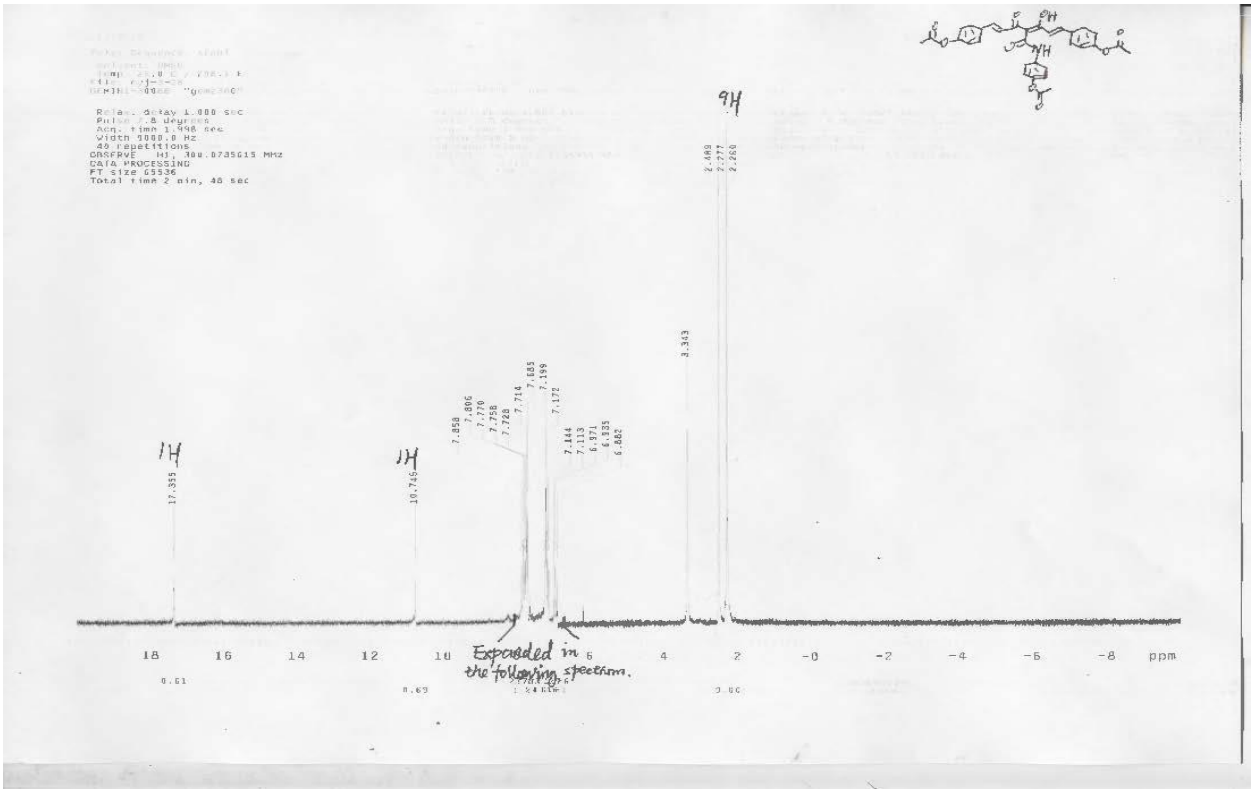
^{1}H-NMR Spectrum of 9c



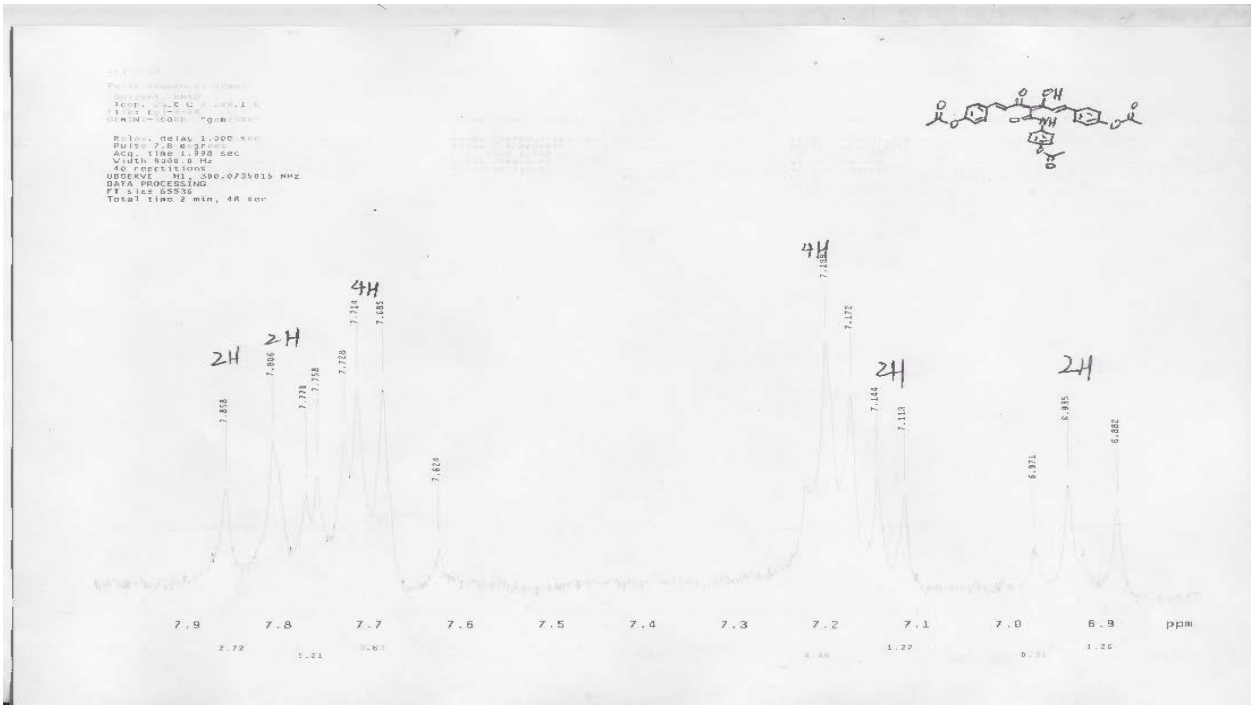
^{1}H-NMR Spectrum (Expanded from δ 8.00~6.80) of 9c



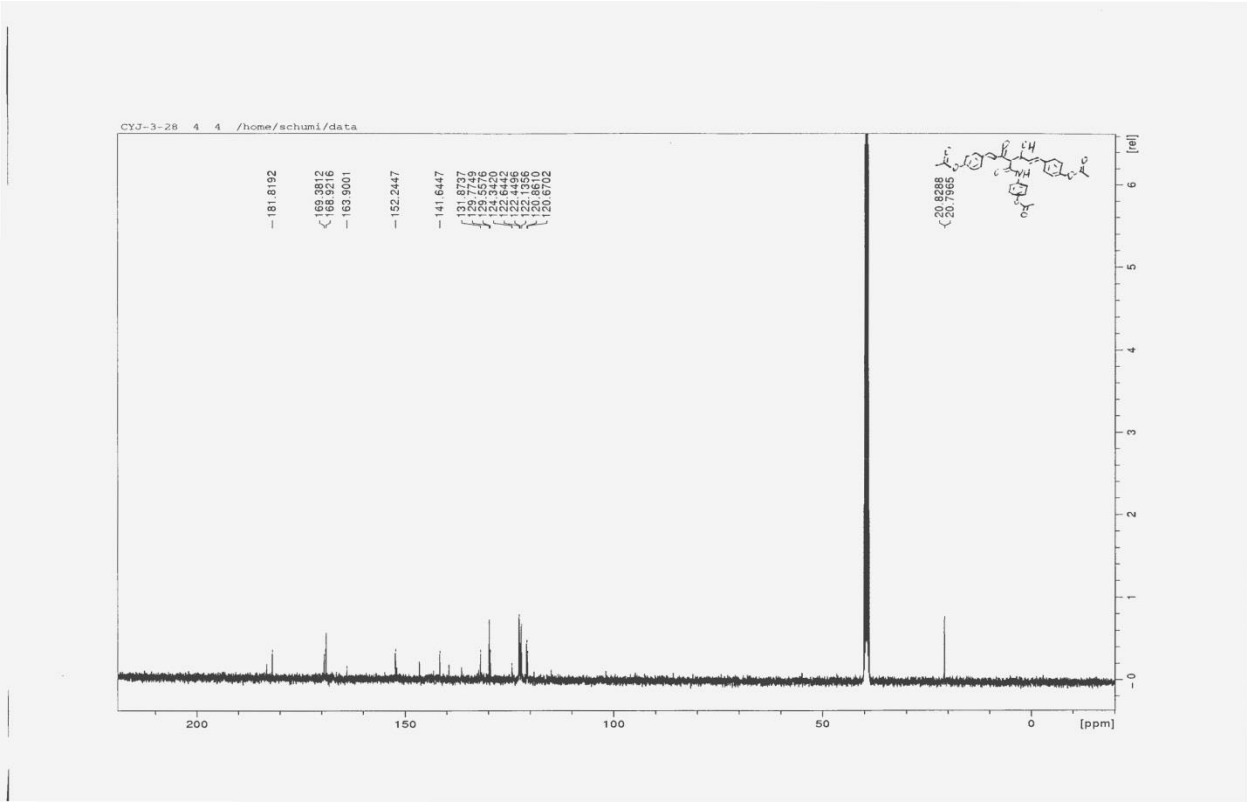
^{13}C-NMR Spectrum of 9c



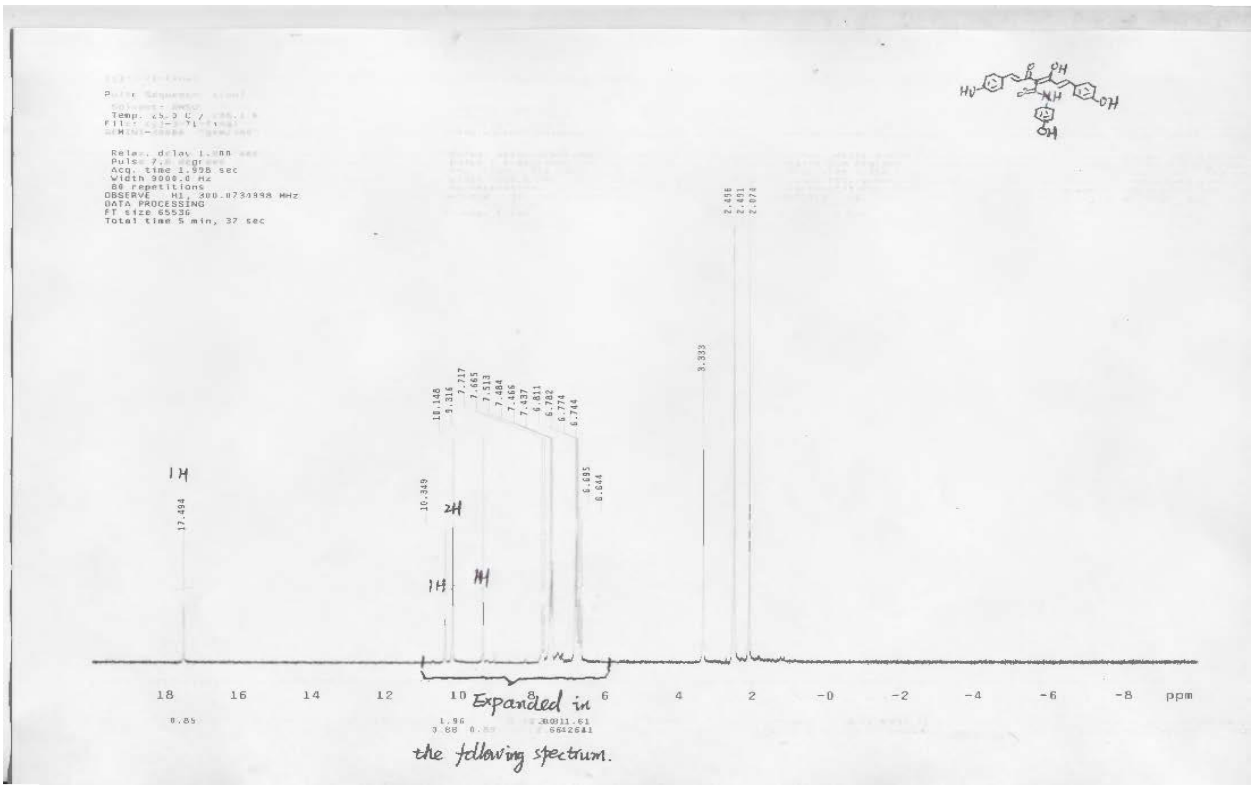
^{1}H-NMR Spectrum of 9d



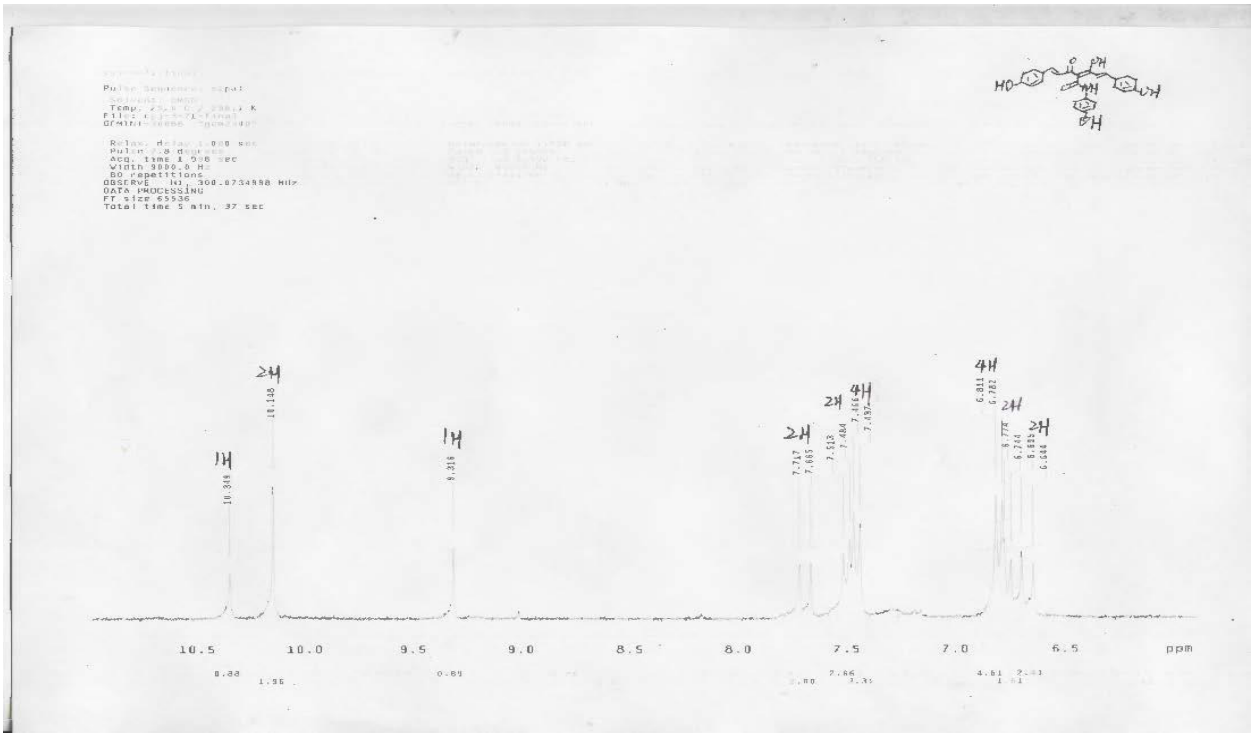
[1]H-NMR Spectrum (Expanded from δ 8.00~6.80) of 9d



[13]C-NMR Spectrum of 9d



^{1}H-NMR Spectrum of 9e



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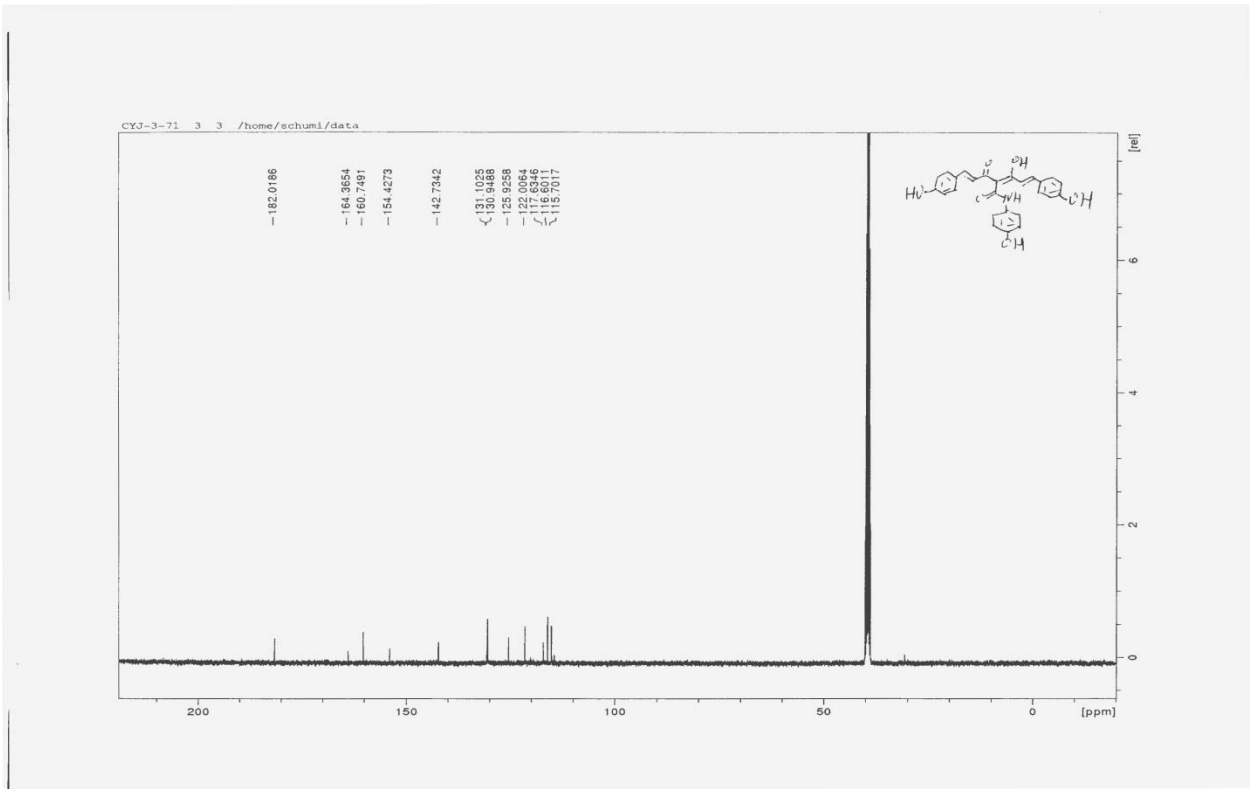
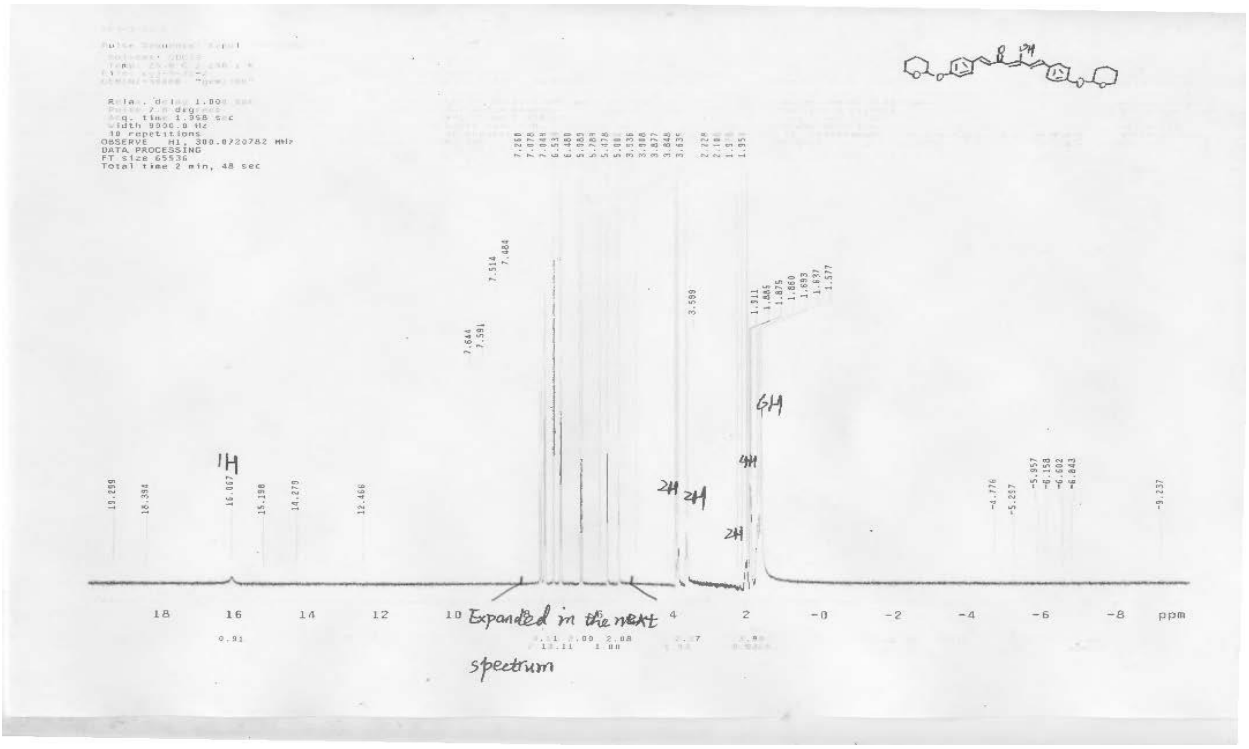
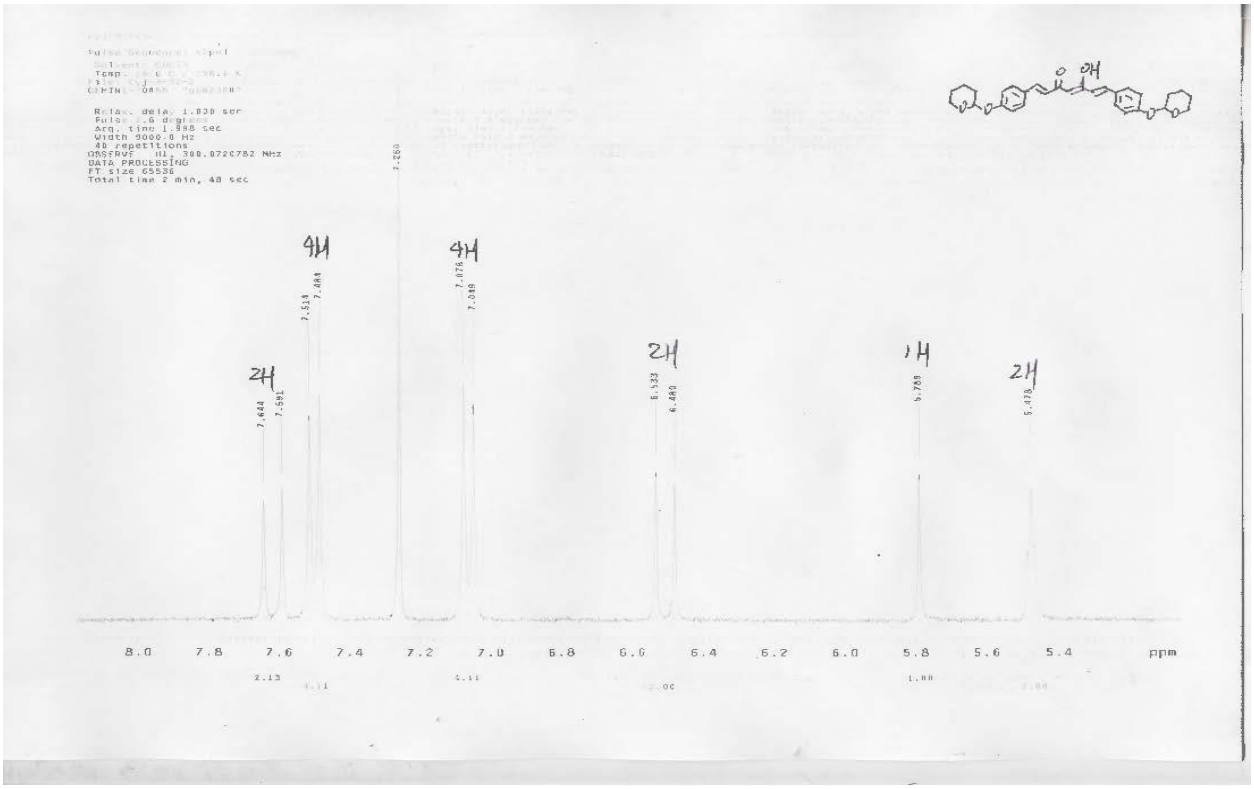


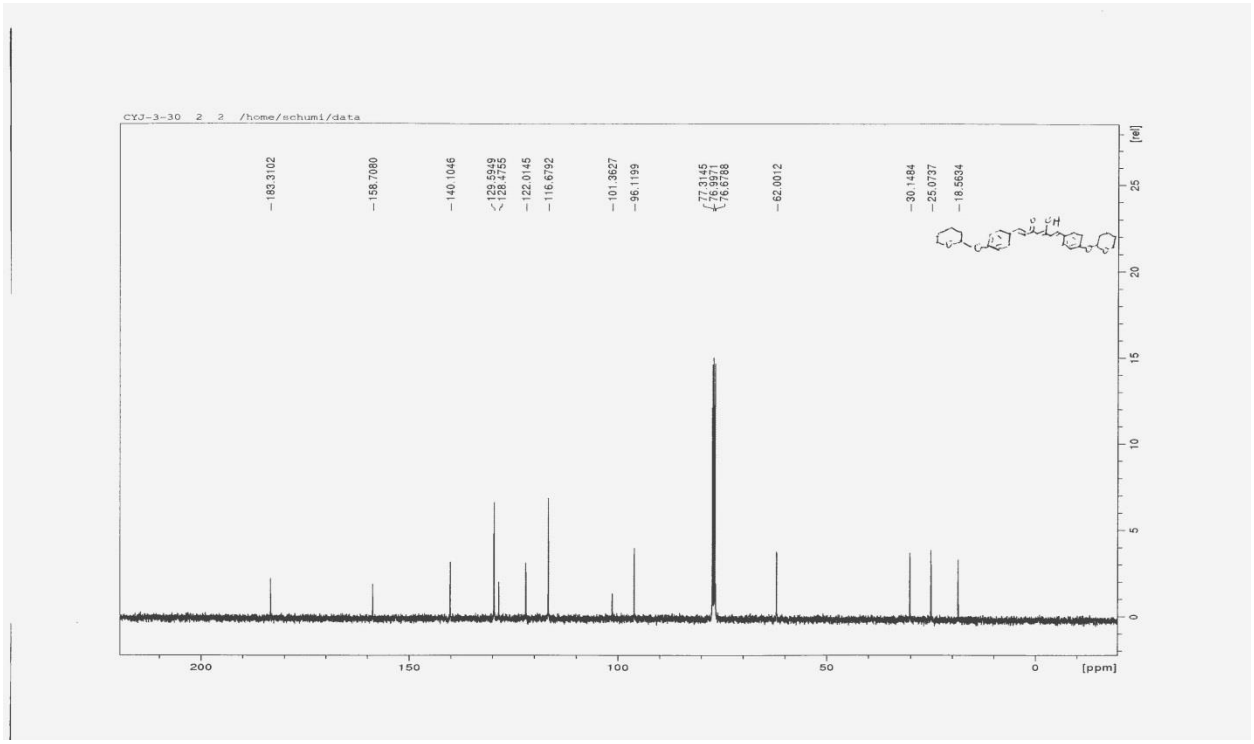
^{13}C-NMR Spectrum of 9e



^{1}H-NMR Spectrum of 9f



^{1}H-NMR Spectrum (Expanded from δ 8.10~5.20) of 9f



^{13}C-NMR Spectrum of 9f