SYNTHESIS OF CAMPTOTHECIN DERIVATIVES

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FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
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Approval of the Graduate School of Natural And Applied Sciences

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We certify that we have read this thesis and in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

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ABSTRACT

SYNTHESIS OF NEW CAMPTOTHECIN DERIVATIVES

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This study presents synthetic studies on camptothecin, a potent antitumor agent in order to improve its stability and solubility without reducing its activity. The study consists of the modification of camptothecin at 20-OH position a new strategy for the targeted and controlled release of the drug and modification at C-7 position to overcome the stability and solubility problems of the free drug.

In the first part of the study, the 20-OH functional group of camptothecin was replaced with an unsymmetrical benzoin derivative that is able to release the drug under photolysis at 350 nm. The new prodrugs synthesized possessed higher stability than the camptothecin itself. The *in vitro* irradiation of the prodrugs at 350 nm was satisfactory without any decomposition of the active substance.

The second part of the study comprises the studies on the modification of the 7^{th} position of camptothecin, which is the most suitable position for the modification. In this

part of the study, 7-amino and silyl substituted camptothecins were synthesized.Combination of camptothecin with some other drugs such as cisplatin was also investigated in this study. The synthetic efforts showed that the reactions are very promising and the combination studies can be studied as a major subject in the future.

Keywords: Camptothecin, unsymmetrical benzoin, prodrug

ÖZ

CAMPTOTHECIN TÜREVLERİNİN SENTEZİ

Duygu, Arife Neşe
Doktora, Kimya Bölümü
Tez Yöneticisi : Prof. Dr. Ayhan S. Demir

Temmuz 2005, 148 sayfa

Bu çalışma , antitümor özelliklere sahip camptothecin molekülünün aktivitesini düşürmeden, kararlılık ve çözünürlüğünü artırmak için yapılan sentetik çalışmaları içermektedir. Çalışma iki bölümden oluşmaktadır. Birinci bölümde CPT`nin 20-OH fonksiyonu türevlendirilerek hedefli ve kontrollü salınım ozelliğine sahip yeni on ilaçlar sentezlenmiştir.Ikinci aşamada ise C-7 pozisyonu türevlendirilerek CPT`nin kararlılık ve düşük çozünürlük sorunları aşılmaya çalışılmıştır.

Çalışmanın ılk bolümünde, 20-OH grubu, simetrik olmayan çeşitli benzoin türevleriyle yer değiştirmiştir.Kullanılan benzoin türevlerinin yaklaşık 350 nm de bağlı oldukları grubu salma ozellikleri vardır.Sentezlenen ürünlerin camptothecine gore daha kararlı olduğu gosterilmiştir.Deneysel ortamda yapılan fotosalım reaksiyonları başarılı sonuçlar vermiştir.

Çalışmanın ikinci bolümü, modifikasyon için en uygun pozisyonlardan biri olan C-7 nin türevlendirilmesi çalışmalarını içermektedir.Bu kapsamda 7 pozisyonu silil-

amin grupları ile türevlendirilmiştir.Bunun yanında CPT ile cisplatin ve benzeri ilaçlarin kombinasyonu üzerine çalışmalar yapılmıştır.

Anahtar kelimeler: Camptothecin, simetrik olmayan benzoinler, önilaç

To My Family,

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Finally, I wish to thank my parents for their encouragement, patience and love.

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LIST OF ABBREVIATIONS

CPT	:	Camptothecin
FDA	:	Food and Drug Administration
TOPO-I	:	Topoisomerase I enzyme
SDS	:	Sodium dodecyl sulfate
SUMO	:	Small ubiquity modifiers
SR	:	Serine-arginine
SF_2/ASF	:	Splicing factor/ Alternative splicing factor
TRFp	:	Topo related function protein
DHQD	:	Dihydroquinidine
PEG	:	Polyethyleneglycol
PHPMA	:	Poly(N-(2-hydroxypropyl)methacrylamide)
TMS	:	Trimethylsilyl
DMSO	:	Dimethylsulfoxide
DMF	:	Dimethylformamide
HAS	:	Human Serum Albumin
TLC	:	Thin Layer Chromatography
THF	:	Tetrahydrofuran
NMR	:	Nuclear Magnetic Resonans
HPLC	:	High Performance Liquid Chromatography
DMAP	:	Dimethylaminopyridine
TFA	:	Trifluoroaceticacid
DBU	:	Diazabicycloundecene
TEAA	:	Triethylamine-acetic acid

CHAPTER 1

INTRODUCTION

1. 1 Cancer and cancer chemotherapy

Cancer is one of the leading causes of death in the industrialized world. Cancer arises when a population of cells within the body escapes from normal control mechanisms and continues to increase until, unless effectively treated, the host dies. Cancerous growths may be controlled by surgical removal, ionizing irradiation (radiotherapy), or chemotherapy. Surgery and radiotherapy are only successful if the lesion is still localized so that the tumor can be either completely excised or covered by a field of irradiation. However, these methods become much less effective for cancers at later stages. Chemical methods can be used for both local and metastatic lesions by interfering with cell division, thereby slowing down or reversing the growth of the cancer.

Since the discovery of mustard gas as the first anticancer agent, many anticancer compounds with different modes of action have been discovered and studied. They can be roughly classified as follows:

i) Alkylation agents: This class of agents forms covalent bonds with biologically important molecules, for example, formation of cross linkages between DNA, thus blocking pathways for cell mitosis. An example is nitrogen mustards.

ii) Antimetabolites: This class of compounds has similar chemical structures to certain essential metabolites. They bind to nuclear material or vital enzymes in an irreversible fashion, thereby inhibiting any further biological activity. Examples of this class of agents are 5-fluorouracil, cytosine arabinoside and 6-thioguanine.

iii) Antimitotic agents: This class of compounds interferes with the microtubule system of cells. By either stabilizing microtubule, such as taxol, or destabilizing microtubule, such as vinblastine, this family of agents can interrupt the tubulin microtubule equilibrium, and thus prevent cell division.

iv) Anticancer antibiotics: Besides their ability to inhibit the multiplication of bacteria, some antibiotics have been found to have similar inhibitory effect on tumor cells. Anticancer antibiotics can be divided into two groups, the anthracycline antibiotics and the non-anthracycline antibiotics.

v) Miscellaneous agents: Some compounds are not as clearly classified as above. Those compounds include non-classical alkylating agents, anthracenediones, epipodephyllo-toxins, hydroxyurea, amsacrine and enzyme therapy agents.

Besides the anticancer agents mentioned above, further studies of the biochemistry of cancer have been revealing new targets for chemotherapy, thus stimulating the development of new anticancer agents with novel mechanism of action. One such example is the topoisomerase I inhibitor camptothecin. The unique mechanism of action of camptothecin makes it the parent of an important class of anticancer agents.

1.2 Camptothecin

Camptothecin (CPT) (1) (Figure 1) is a potent antitumor antibiotic isolated by Monroe E. Wall and Mansukh C. Wani in 1958 from extracts of Camptotheca acuminata, a tree native to China and Tibet which has been extensively used in

traditional Chinese medicine.[1] The structure was determined to be that of a pentacyclic alkaloid and was first reported in 1966.[2]

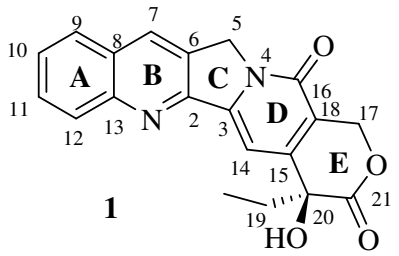


Figure 1: Camptothecin

The success of CPT in preclinical studies led to clinical investigations. Due to the negligible water solubility of CPT, these trials were initiated using the water-soluble sodium salt (2). The lesser efficacy of the sodium salt, accompanied by unpredictable and severe levels of toxicity associated with treatment, including hemorrhagic cystitis and myelotoxicity, resulted in suspension of the trials.[3]

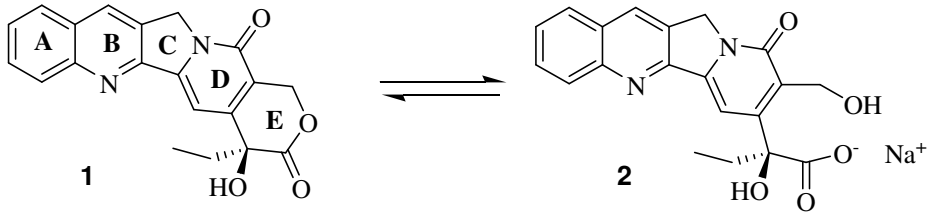
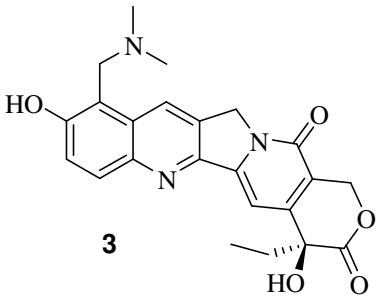


Figure 2: Camptothecin hydrolysis to water soluble sodium salt

The discovery that the primary cellular target of CPT is type I DNA topoisomerase (topo) created renewed interest in the drug.[4] Advances in the medicinal chemistry of CPT resulted in the semi-synthetic, more water soluble analogues topotecan (Hycamtin, 3) (Figure 3) and irinotecan (Camptosar, 4) (Figure 4).

Topotecan (9-[(dimethylamino) methyl]-10-hydroxy-camptothecin) was the first camptothecin analogue to be approved for clinical use by the US Food and Drug Administration (FDA). It is water-soluble because of its side-chain at the 9th carbon of the A ring. Results of preclinical studies suggested topotecan to have excellent antitumor activity in vitro. Tumor xenograft models showed activity in many tumor types, including adenocarcinomas of the ovary and colon, tumors of the central nervous system, and sarcomas. [5]



3

Figure 3: Structure of topotecan

Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy camptothecin) was the first water-soluble semisynthetic derivative of camptothecin to enter clinical trials. A unique characteristic of irinotecan is its bulky dipiperidino side-chain linked to the camptothecin molecule via a carboxyl-ester bond (figure 4). This side-chain, although providing necessary solubility, leads to a substantial reduction in anticancer activity. Cleavage of the side-chain by carboxylesterases—found mainly in the liver and gastrointestinal tract—forms the metabolite SN-38 (5) (7-ethyl-10-hydroxycamptothecin). Irinotecan became commercially available in Japan in 1994, where its approved indications were cancers of the lung (small-cell and non-small-cell), cervix, and ovaries. It was approved in Europe in 1995 as a second-line agent for colon cancer, 1 year before European approval of topotecan. Irinotecan was approved in the USA in 1996 for treatment of advanced colorectal cancer refractory to fluorouracil. Additional CPT analogues are also under investigation, and are also of interest in combination regimens as radiation sensitizers.[6]

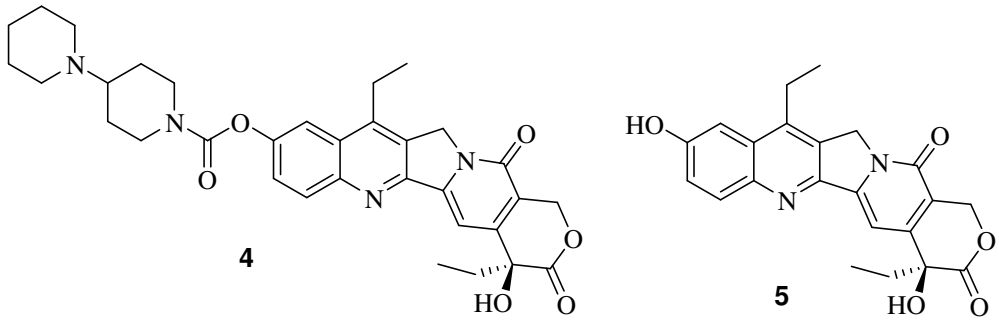


Figure 4: Structures of irinotecan and SN-38

1.2.1 DNA Topoisomerases

DNA topoisomerases are a unique class of enzymes that change the topological state of DNA by breaking and rejoining the phosphodiester backbone of DNA.[7] Based on fundamental differences in their reaction mechanisms; DNA topoisomerases are classified into two types. Type I DNA topoisomerases change the topological state of DNA by transiently breaking one strand of the DNA double helix and therefore characteristically change the linking number of DNA in multiplies unity. Type II DNA topoisomerases catalyze the strand passing reaction by making transient, enzyme bridged, double strand breaks and consequently change the linking number of DNA in multiplies of two.

Human DNA topoisomerases are the archtype of mammalian DNA topoisomerases. Human Topo I is a monomeric protein, encoded by a single-copy gene located on human chromosome 20q12-13.2. Human Topoisomerase II on the other hand, is a homodimeric protein, encoded by a single copy gene on human chromosome 17q21-22. Both Topo I and topo II catalyze the relaxation of supercoiled chromosomal DNA during DNA replication. The relaxation of DNA by topo II involves the transient double-strand breakage of DNA, followed by strand passage and religation of the DNA strands. Topo II relaxation of DNA requires ATP and results in a change in linking

number by (multiples of) two. In contrast, the mechanism of DNA relaxation by topo I involves the transient single strand cleavage of duplex DNA, unwinding and religation. Specifically, topo I mediated DNA strand scission involves a nucleophilic attack by the active site tyrosine OH group on the DNA phosphodiester at the site of cleavage (Figure 5). This attack results in breakage of the DNA phosphodiester backbone and formation of a phosphorotyrosine bond between the enzyme and DNA; the resulting intermediate is termed the 'covalent binary complex'. Following DNA relaxation via passage of the broken DNA strand around the unbroken strand, the phosphodiester backbone is reformed by religation with concomitant release of the enzyme. Inhibitors of both topo I and topo II are characterized by their ability to stabilize the covalent binary complex, thus diminishing religation and ultimately DNA synthesis and cell viability. [8]

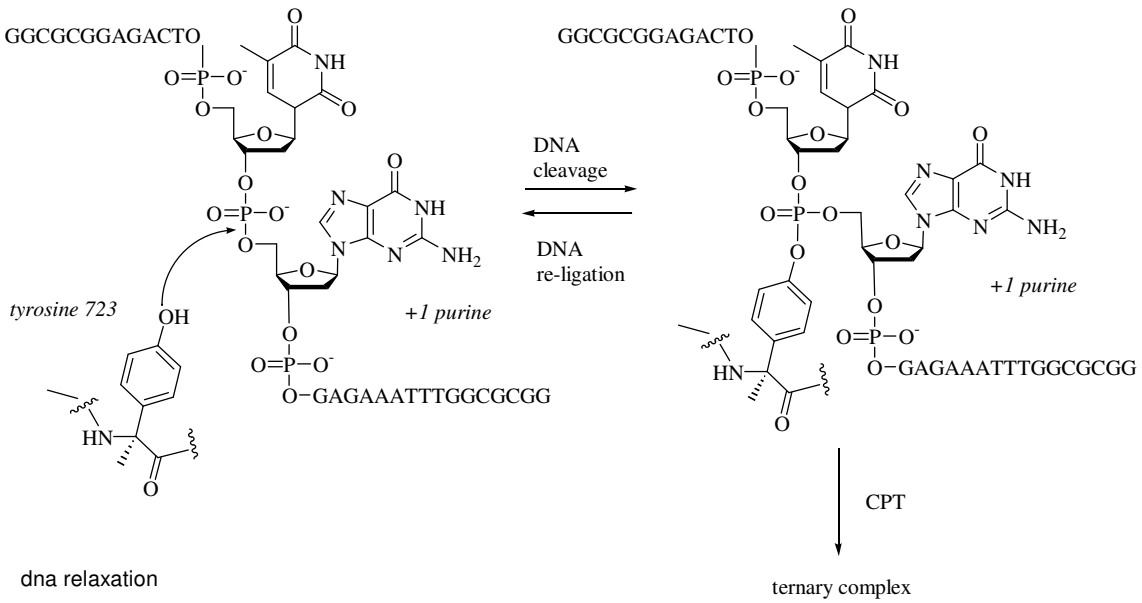


Figure 5: Mechanism of DNA relaxation by topoisomerase I

1.2.2 Camptothecins as Topoisomerase Poisons: Mechanism of Action

Medicinal agents believed to function at the locus of topo II include a number of clinically used anti-neoplastic agents such as doxorubicin and mitoxantrone.[9] Numerous topo I inhibitors have been characterized, including rebeccamycin, indolocarbazole, nitidine and 2-phenylbenzimidazole.[10] The CPT family, however, remains the most extensively studied class of agents that target topo I.

CPT is a highly phase-specific cytotoxic drug; S-phase cells are selectively killed. Like topoisomerase II poisons, camptothecin also induces sister chromatid exchanges and chromosal aberrations. In cultured mammalian cells, it strongly inhibits both DNA and RNA synthesis, and induces fragmantation of chromosomal DNA. Both inhibition of RNA synthesis and fragmantation of chromosomal DNA are rapidly "reversible". Inhibition of DNA synthesis, on the other hand, is only partially reversible. CPT does not bind to or react with purified DNA. Neither does CPT inhibit purified RNA or DNA polymerases. These results led to the suggestion that a cellular target might mediate the action of CPT. [8]

CPT was initially tested for its possible effect on mammalian DNA topoisomerase II. Surprisingly, CPT had no inhibitory effect on purified DNA topoisomerase II. However, it produced large amounts of single-strand DNA breaks in the presence of topoisomerase I. Topoisomerase I was covalently linked to the 3' phosphoryl end of the broken DNA strand. Topoisomerase I-mediated DNA cleavage in the presence of CPT is rapid and prolonged incubation does not lead to more cleavage. In fact, less DNA cleavage is normally observed with prolonged incubation when the lactone form of CPT is used, presumably due to the hydrolysis of lactone to the inactive sodium form. Treatment of the reaction mixture with a strong protein denaturant, such as SDS or alkali, is required to induce topoisomerase I-mediated DNA strand breaks. If prior to treatment with a strong protein denaturant, the reaction is diluted, challenged with excess second DNA, or adjusted to 0.5 M NaCl, DNA cleavage is greatly reduced.

Together, these results suggest that CPT interferes with the breakage reunion reaction of DNA topo I by trapping the key covalent reaction intermediate, the cleavable complex. The cleavable complex, which is productive reaction intermediate in the relaxation reaction and is present in small amounts, is in rapid equilibrium with the noncleavable complex. In the presence of CPT, the cleavable complex is stabilized and becomes nonproductive in the relaxation reaction. However, CPT-stabilized cleavable complexes are still in rapid equilibrium with noncleavable complexes. The exposure of drug stabilized cleavable complexes to a strong protein denaturant leads to topo I-linked, single strand breaks. Based on this proposed mechanism, CPT was considered as a topo I poison. The majority of CPT-stimulated DNA cleavage sites occur at position similar to the background cleavage sites of topo I in the absence of CPT.[11]

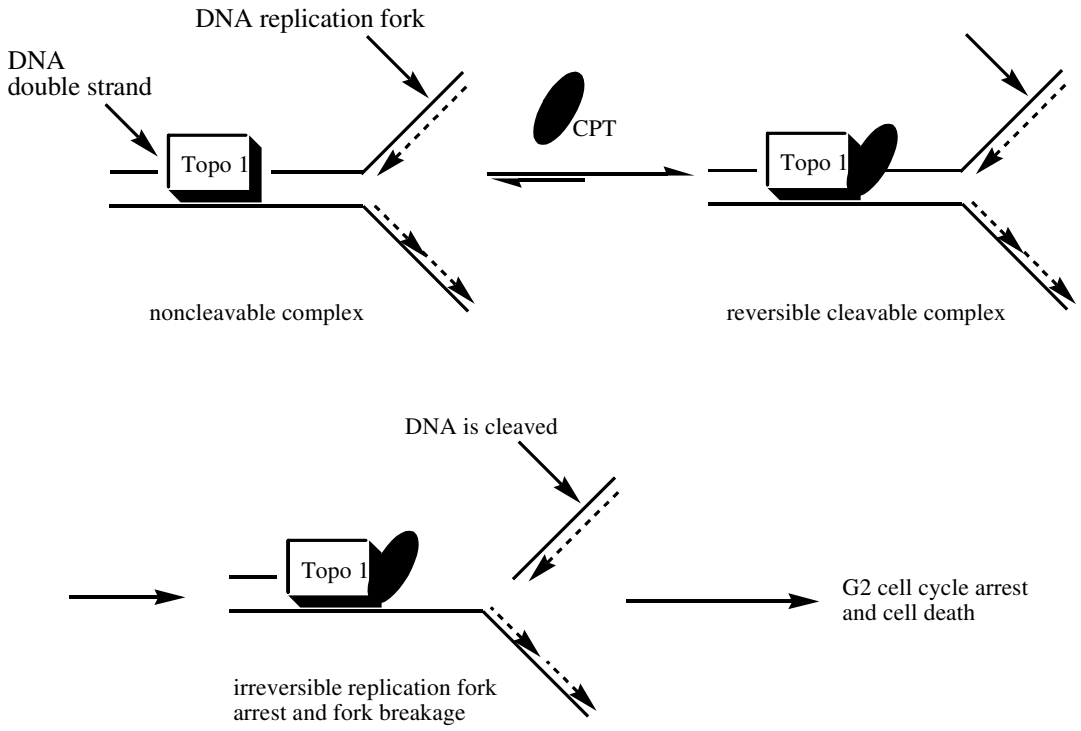


Figure 6: Mechanism of action of Camptothecin

1.2.3 Structural Models for the Ternary Cleavable Complex Formed Between Human Topo I, DNA, and CPT

There are several structural models suggesting for the interaction of CPT with the covalent binary complex. The recent binding models were greatly facilitated by X-ray crystal structures of topo I interacting both covalently and noncovalently with DNA.[23] Kerrigan et al. derived a ternary 20(*S*)-CPT-DNA-TOP1 cleavable complex structure suggesting that Arg_{364}, Asp_{533}, and Asn_{722} are the closest amino acid residues to the intercalated drug. (Figure 7) Arg_{364} and Asp_{533} lie in the DNA minor groove, while Asn_{722} lies on the major groove side of the cleavage site. These amino acid residues, as well as the -1 and +1 base pairs of the DNA, represent the potential sources for favorable interactions with the bound drug. The hydrogen bonding interactions (using a distance cutoff of 3.1 Å) of 20(*S*)-CPT with both the DNA and topo1 are also schematically presented in Figure 7. Inspection of this figure reveals that 20(*S*)-CPT stabilizes the cleavable complex primarily through hydrogen bonding interactions involving its E- and B-rings. Specifically, both the O atom and the 21-carbonyl oxygen of the E-ring make multiple hydrogen bonding contacts with Arg_{364}. In addition, the 20-hydroxyl proton is capable of making a hydrogen bond with either the N3 atom of the +1 guanine or a DNA furanose ring oxygen. The N1 atom on the B-ring of 20(*S*)-CPT makes hydrogen bonding contacts to both the 3NH proton of the -1 thymine as well as the 6-NH_2 group of the adenine complement to the -1 thymine. In addition to these hydrogen bonding interactions, stacking interactions between the C-ring of the drug and the adenine complement to the -1 thymine also contribute to the stability of the ternary cleavable complex. In the aggregate, these observations suggest that 20(*S*)-CPT stabilizes the TOPO1-DNA cleavable complex through an array of van der Waals and hydrogen bonding interactions with both the enzyme and the DNA. [12]

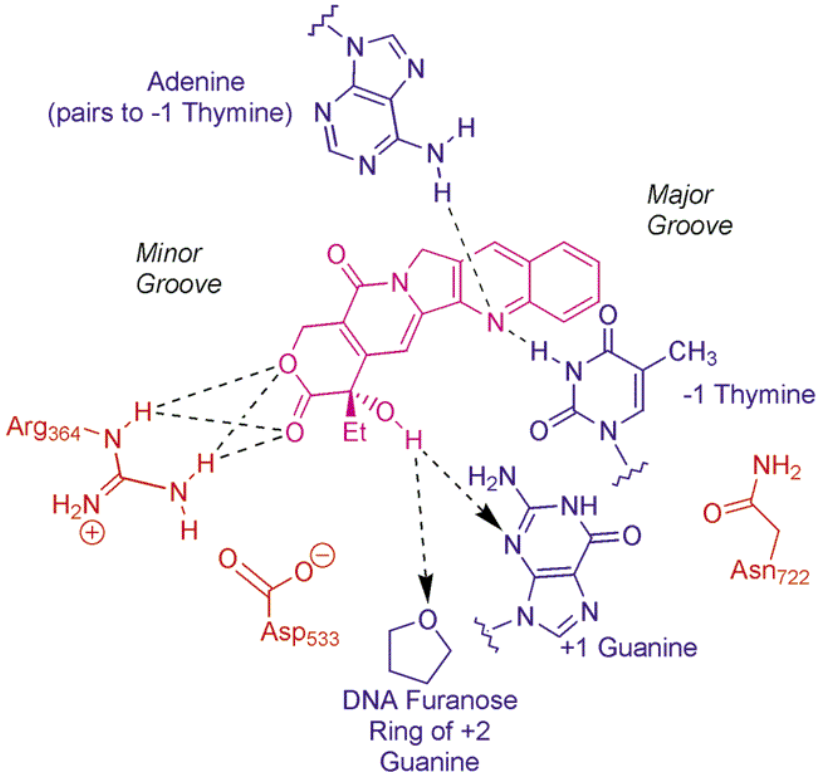


Figure 7: Structure model for the ternary complex between DNA-topo I and CPT

As it can be seen from the figure, the A-B ring of the molecule is suitable for modification it is indeed provide wide possibilities of variation, especially in positions 7,9,10 and 11.

1.2.4 Other Biochemical Effects of CPT

A number of experiments have suggested that CPT induced, topo I-DNA cleavable complexes are responsible for most but not all the biological effects of CPT. The ability of CPT to convert topo I into a cellular poison is believed to be contingent upon the trapping of the covalent binary complex, which results in DNA damage during the S-phase of the cell cycle. It has been reported that elevated levels of topo I exist in tumors of the colon, ovary and prostate, which could well result in enhanced sensitivity

toward CPT.[13] Also suggested as a source of selectivity has been deficiencies in metabolic pathways within cancer cells that result in the impairment of normal DNA repair mechanisms.[14]

A number of experiments have suggested that CPT induced, topo I-DNA cleavable complexes are responsible for most but not all the biological effects of CPT. Further, in addition to relaxation of supercoiled chromosomal DNA, topo I participates in other cellular functions that are sensitive to CPT.[15]

It has been reported that CPT induced rapid conjugation of ubiquity and small ubiquity modifiers (SUMO) to topo I, resulting in subsequent protein degradation. CPT, therefore, activates a cellular response capable of degrading, and thus potentially resolving, the trapped covalent binary complex. However, it has also been suggested that other cellular processes may also be affected by CPT, leading to modulation of the effects of CPT.[16]

Tazi et al. have demonstrated that topo I possesses activity as a phosphorylating enzyme for SR proteins and, by extension, influences the splicing activity of SR proteins.[17] The kinase activity of topo I utilizes ATP as the phosphate donor and has been shown to be separate from its DNA relaxation function through mutational studies.[18] Despite this separation of activities, the kinase function of topo I is inhibited by CPT in the presence of DNA. Interestingly, the splicing factor SF_2/ASF, a phosphorylation substrate for the kinase activity of topo I, was shown to be capable of inhibiting CPT induced DNA cleavage in a cell free system.[19]

The identification of a series of topoisomerase I related function proteins (TRFp) has been reported by Christman et al. Analysis shows that one member of this family, TRF4p, plays a critical role during the S phase of the cell cycle. Specifically, in conjunction with topo I, TRF4p is involved in mitotic chromosome condensation. Additionally, TRF4p is associated with the DNA binding protein Smc1p during chromosomal condensation. Importantly, reports detailing mutations to TRF4p have

produced cell lines with surprising hypersensitivity toward CPT, suggesting that TRF4p may be important for repairing DNA damage caused by CPT. The TRF4p locus has recently been shown to encode a novel DNA polymerase, presently denoted polymerase. The effects of inhibition of this enzyme on cellular sensitivity to CPT seem important to establish. [20]

In addition to its DNA relaxation activity and the other functions described above, topo I is able to regulate transcription, recognize and cleave mismatched nucleotides at intrinsic cleavage sites and associate with numerous proteins in vivo. Topoisomerase I is also implicated in non-homologous recombination, which could well be linked to the process of tumor genesis. It has also been reported that irinotecan inhibits acetyl cholinesterase activity.[21] Inhibition of acetyl cholinesterase could obviously constitute a dose-limiting factor for utilization of the drug. While the reports described above represent a solid foundation, a through understanding of the manner by which CPT may affect cellular function at loci other than topo I is still in its infancy.

1.2.5 Synthetic Studies

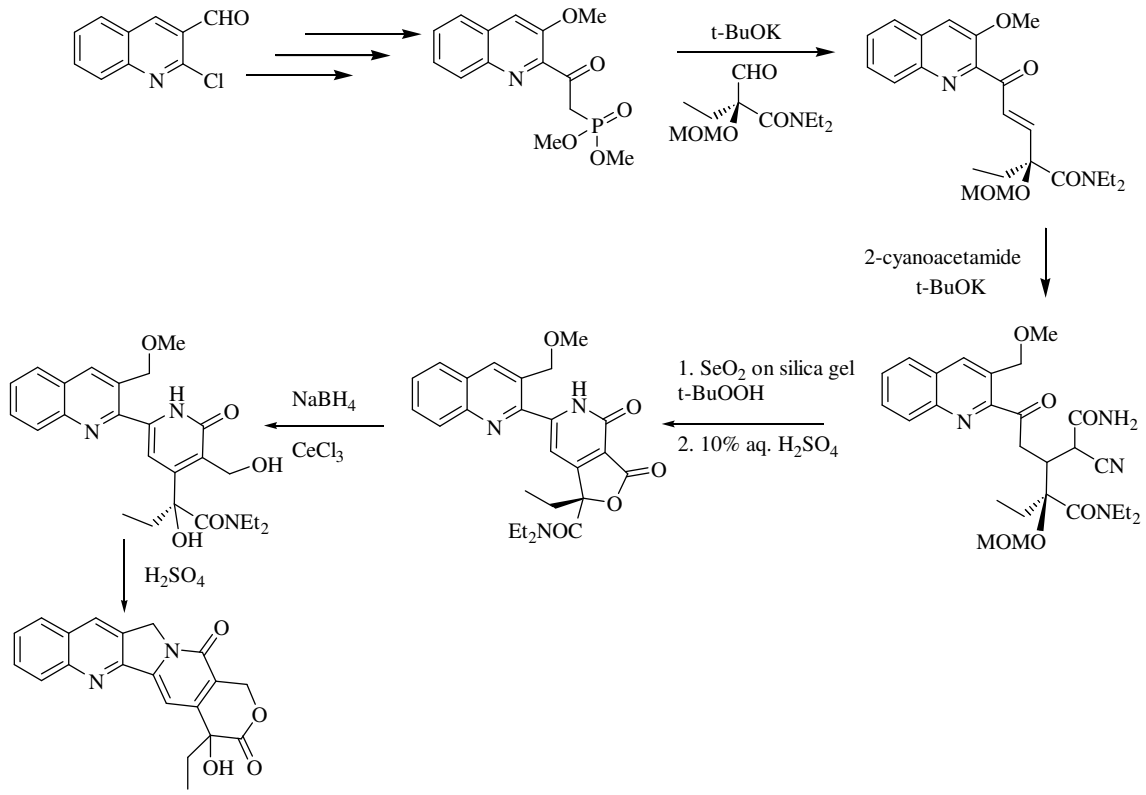
Today, CPT analogues with improved therapeutic properties are of great interest for both understanding the mechanism of topo I interaction in greater detail, and also their ability to overcome CPT application problems. There are now several synthetic approaches to CPT and its analogues. There are also semi-synthetic approaches for the production of derivatives of CPT.

1.2.5.1 Total Synthesis of Camptothecin

After the initial description of the isolation of CPT, a large number of synthetic efforts were reported utilizing a multitude of approaches.[22] In 1971, Stork and Schultz were reported first successful synthesis of the racemic form of the molecule.[23] Many different synthesis of 20(*RS*)-CPT have since been published.[24]

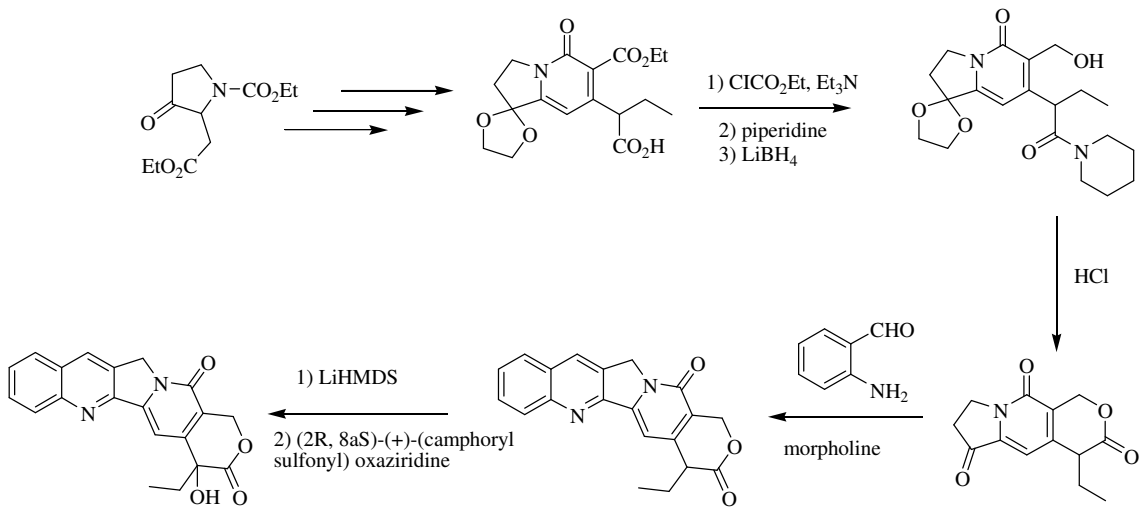
20(S)-CPT was first synthesized by a resolution via quinine of a chiral intermediate by Corey et al. in 1975.[25] The successful resolution of the R and S enantiomers of CPT using (*R*)-(+)-a-methylbenzylamine has also been reported.[26] Recently, Imura et al. described the first asymmetric synthesis of a key chiral intermediate using enzyme-mediated resolution.[27]

The first asymmetric synthesis of 20(S)-CPT was reported by Tagawa et al. in 1989 utilizing an N-tosyl-R-proline derivative as a chiral auxiliary to induce the stereocontrolled assembly of the lone chiral center.[28] Fang et al.[29] and Jew et al. [30] used the chiral catalyst $(DHQD)_2$-pyr for the stereocontrolled oxidation of carbon 20 in greater than 90% enantiomeric excess as part of the total synthesis of 20(S)-CPT. Comins et al. have utilized chiral auxiliaries to establish the correct stereochemistry for the 20(S)-hydroxyl group.[31] Refinement of their method has culminated in the asymmetric synthesis of 20(S)-CPT in only six steps from commercially available starting materials.[32] Ciufolini and Roschangar reported a synthesis of 20(S)- CPT utilizing an esterase to establish the correct C-20 stereochemistry (Scheme 1).[33]



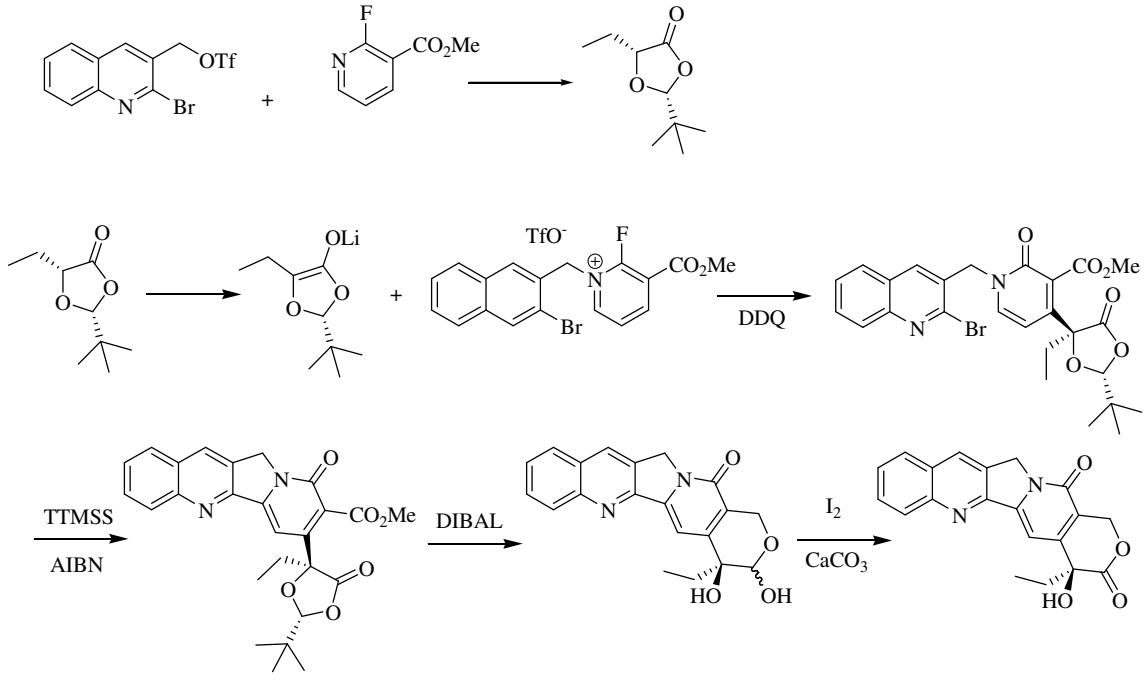
Scheme 1

Nagao et al. have described a novel synthetic strategy leading to 20(S)-CPT from racemic ethyl 1-ethoxycarbonyl-3-oxopyrrolidin-2-ylacetate.[34] Their strategy that is shown in the scheme below is important in that it should be useful for the synthesis of C-20 functionalized CPT analogues.



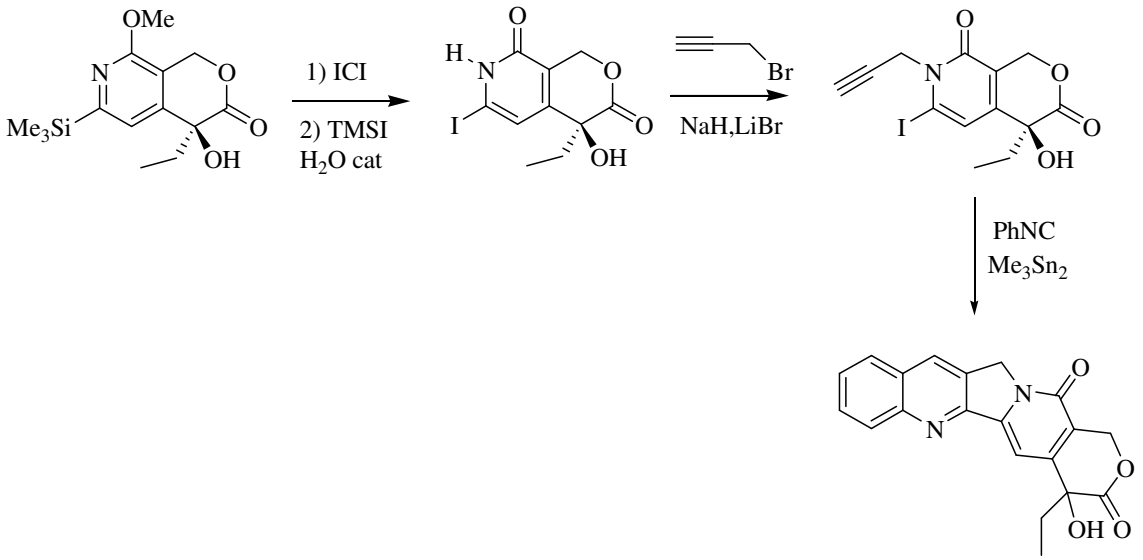
Scheme 2

A recent strategy employed by Bennasar et al. made use of a (2R,5R)-2-tert-butyl-5-ethyl-1,3-dioxolan-4-one to establish C-20 asymmetry and thereby afford access to 20(S)-CPT as it is exhibited in the scheme 3.[35]



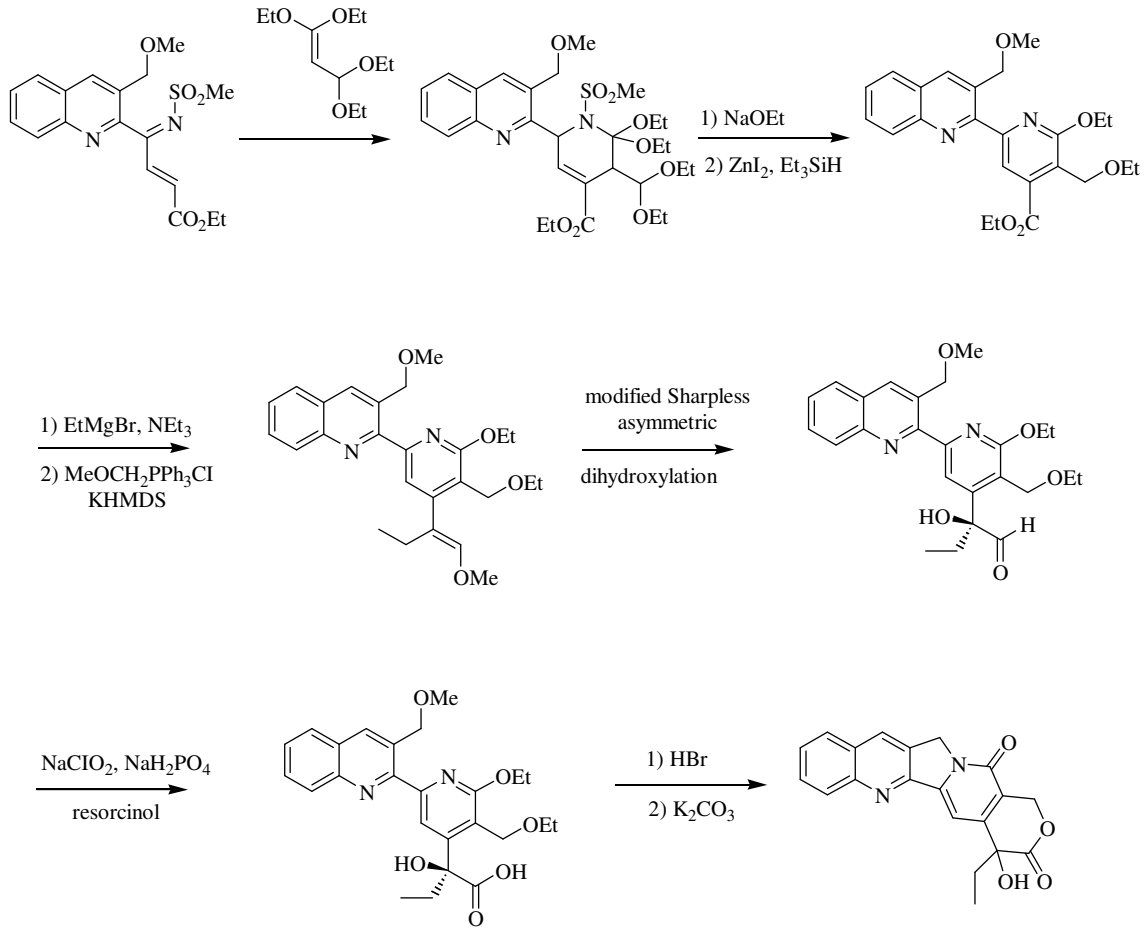
Scheme 3

Curran et al. have reported a synthesis of (S)-CPT based upon an interesting 4+1 radical cascade annulations.[36] In their synthesis lactone was obtained in a fashion similar to the synthesis developed by Fang et al.[29] Their report describes the improvements in the synthesis of the enantiopure DE ring precursor using a samarium catalyst.[37] Radical methods of this type have been shown to tolerate A- and B-ring substituents and have, therefore, been utilized for the synthesis of numerous CPT analogues.[38]



Scheme 4

In another recent report Blagg and Boger described a new synthesis of 20(S)-CPT based on a Diels–Alder cycloaddition of the electron deficient diene N-sulfonyl-1-aza-1,3-butadiene with the electron rich dienophile for the assembly of the precursor to the A, B and D rings (Scheme 5).[39]

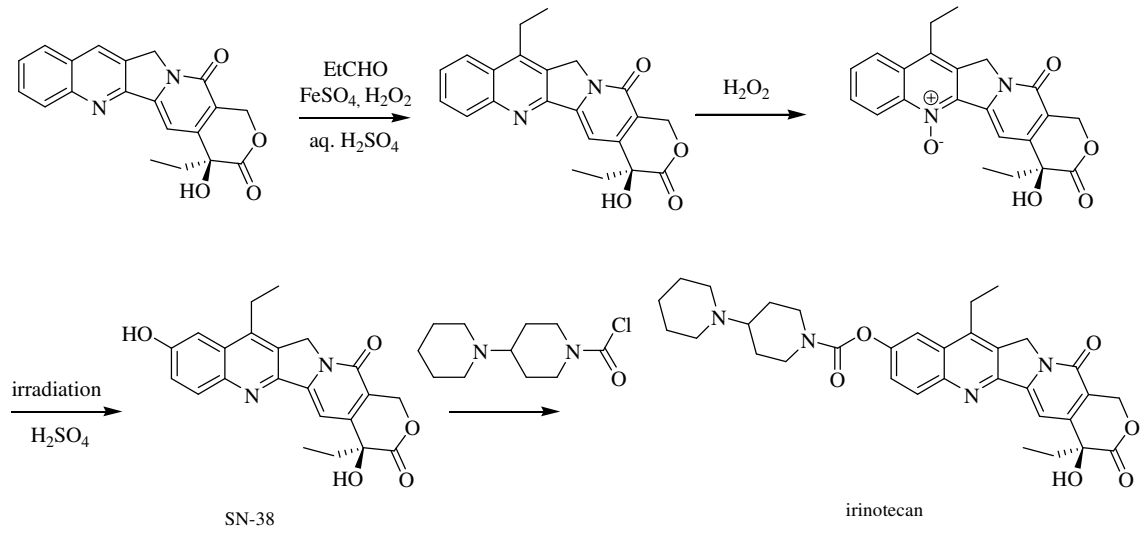


Scheme 5

1.2.5.2 Modifications on Camptothecin Molecules

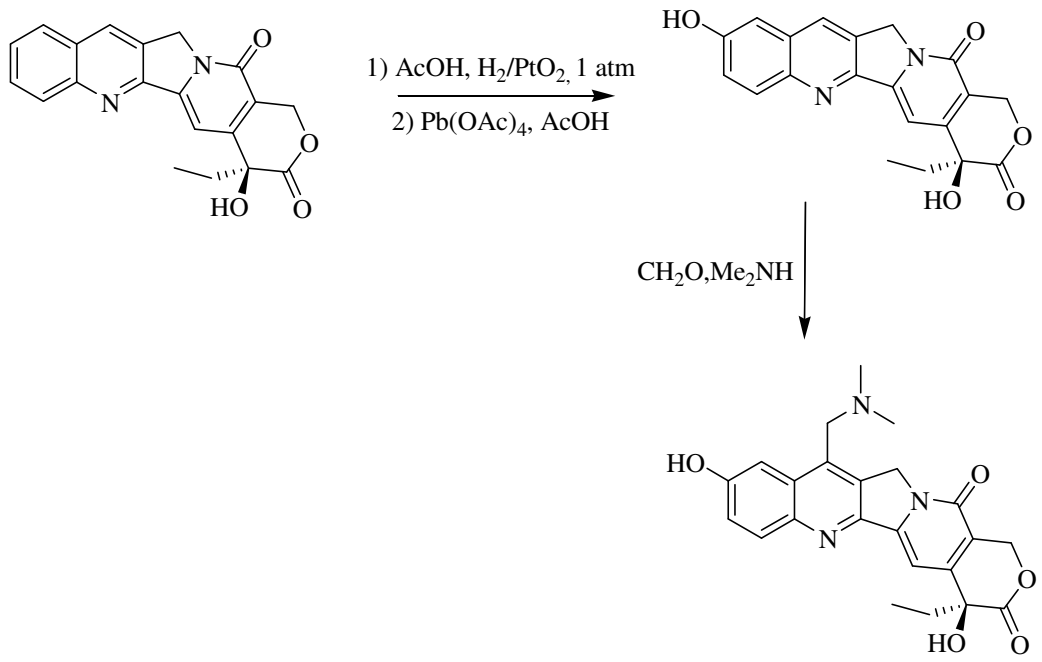
In addition to numerous total syntheses of CPT, many synthetic efforts are aimed at producing analogues. The majority of these efforts have involved the semi-synthetic manipulation of CPT. Among the most important efforts have been the semi-synthetic approaches to irinotecan, topotecan and homocamptothecin. By means of semi-synthetic methods, Sawada et al. reported the synthesis of irinotecan in 1991.[40] (Scheme 6). They used 7-EthylCPT as a starting material and converted into the corresponding N-oxide. Irradiation of the N-oxide in acidic media furnished the active metabolite of irinotecan

(SN-38) in 49% yield. Treatment of SN-38 with 4-(1-piperidino)-1- (piperidino)-chlorocarbamate provided irinotecan in 80% yield.



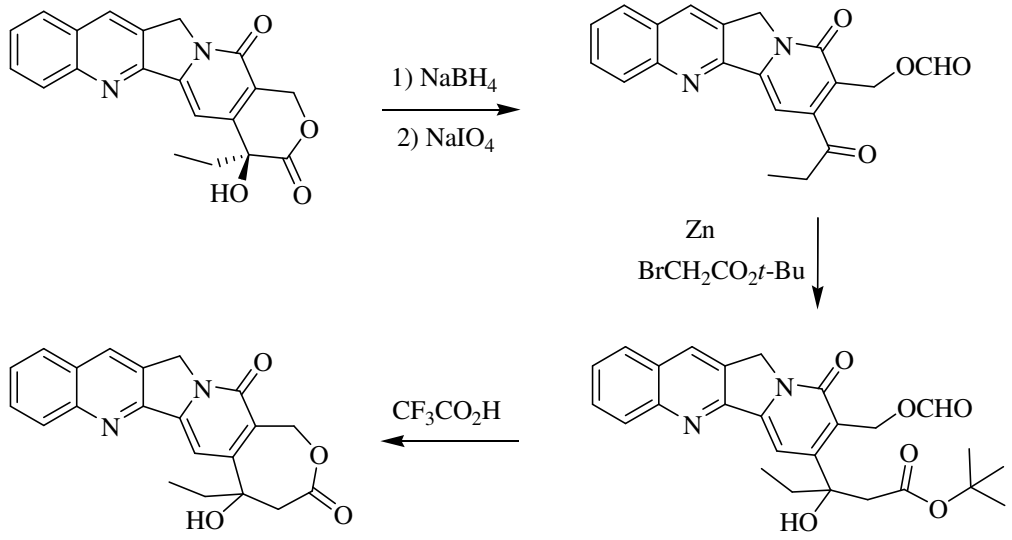
Scheme 6

The second currently marketed CPT derivative, topotecan, was synthesized in 1991 by Kingsbury et al. in two steps starting from 20(S)-CPT.[41] The conversion of 20(S)-CPT to 10-hydroxyCPT was accomplished through a reduction-oxidation sequence and treatment of 10-hydroxyCPT with dimethylamine in aqueous formaldehyde and acetic acid provided topotecan (Scheme 7).



Scheme 7

The parent compound of a new class of CPT derivatives, homoCPT (40), was first prepared in racemic fashion by Bigg et al. utilizing a four-step semi-synthetic sequence (Scheme 8).[42] Curran et al. using the previously discussed 4+1 radical cascade annulations as a key step is also recently reported an asymmetric total synthesis of 20(S) -homoCPT.[43]



Scheme 8

1.2.6 Structure–activity relationships

The development of these synthetic and semi-synthetic strategies has facilitated the study of CPT mechanism, as well as the identification of analogues with improved properties. Likewise, the availability of topo I–DNA and topotecan-topo I–DNA crystal structures and computational docking models provide insight regarding the mechanism of action of CPT, although a number of questions remain unanswered. The latter include an understanding of the exact mode and uniqueness of CPT binding to the enzyme–DNA covalent binary complex and the specific roles of the various structural features of CPT in this critical interaction. Further, it is important to gain an understanding of how systematic modifications within the CPT structure may enhance or suppress the effect of the drug in a biological context.

Numerous studies exploring the structure activity relationships of CPT have provided novel insights and contributed to the clinical successes already realized. Recent studies have explored lactone stabilization, solubility and drug transport mechanisms, tumor cell recognition and enhancement of DNA sequence specificity. The present discussion of CPT derivatives focuses individually upon substitutions, additions and deletions of the quinoline ring system (A/B rings), indolizine moiety (C and D rings), and the E lactone ring of the pentacyclic structure (Figure 8).

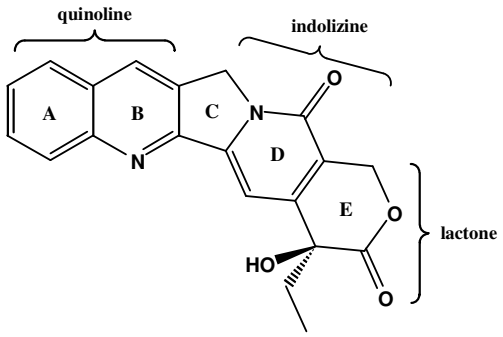


Figure 8: Pentacyclic Structure of camptothecin

At present, the design of novel CPT analogues rests upon a few important working assumptions. The first is that the conjugation and planarity of the A, B, C and D rings are required for in vitro and in vivo activity of the CPTs. Second, it has been thought that the E ring lactone is a necessary structural feature, as the corresponding sodium salt exhibits much lower activity; finally, the S configuration at carbon 20 is believed to be essential for activity, as racemic forms of CPT (analogues) have repeatedly been found to have lesser potency than the optically pure forms.[44]

To date, the only CPT analogues approved for clinical use, that is, topotecan and irinotecan, are derivatives with substitutions within the quinoline ring. Indeed, the A and B rings of CPT have proven to be the most amenable to modification without perturbation of activity. Modifications can involve additions to the quinoline ring or the complete replacement of the quinoline ring with an alternative ring system. Reports detailing analogues that replace the quinoline ring system with alternative conjugated, planar heterocycles suggest that some maintain moderate to good activities.[45] However, none of these derivatives surpasses CPT in potency of action. Consequently, the vast majority of studies have focused upon additions to the quinoline ring and the effectiveness of substitutions at each of the carbon atoms of the quinoline ring (Figure 9).

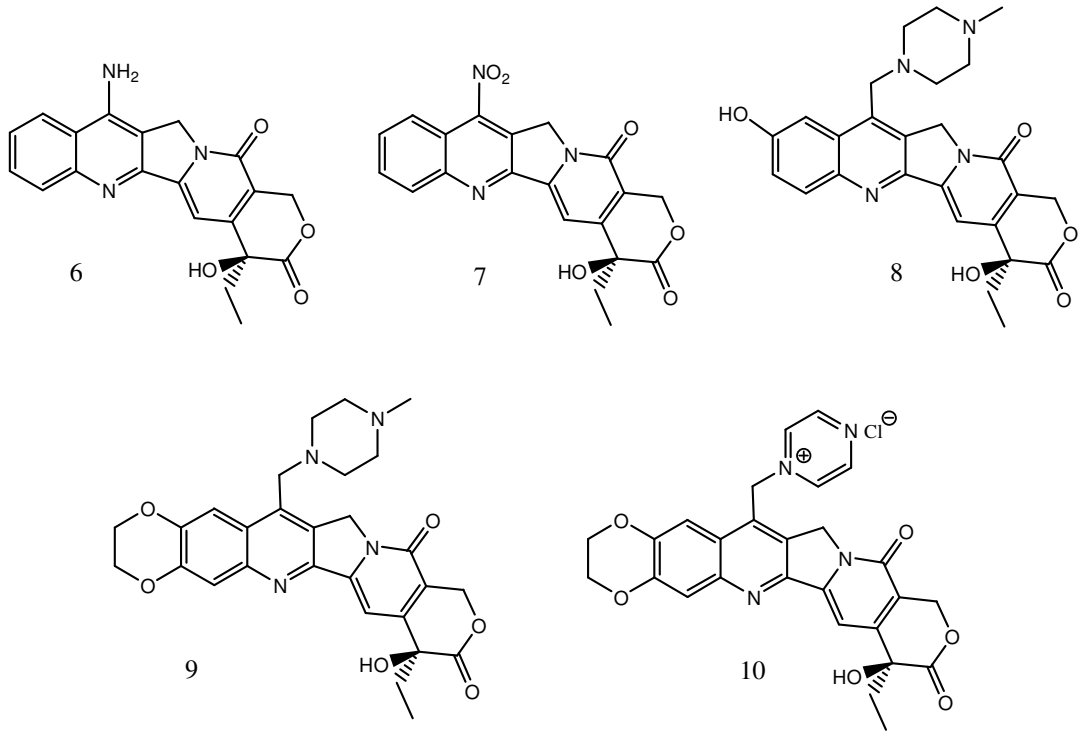


Figure 9: Quinoline ring modified camptothecins

In seminal work, Wani et al. described CPT analogues that afforded a preliminary understanding of the structure activity relationships profile of CPT. Included in this foundation was the finding that substitution at carbons 11 and 12 was generally unfavorable to biological activity. Notable exceptions are analogues containing the 10,11-(methylenedioxy) or 10,11-(ethylenedioxy) functional groups, and also 11-fluoro and 11-cyano derivatives, which enhanced topo I inhibition. Numerous substitutions of amino, halogeno and hydoxy groups at positions 9 and 10 were observed to have beneficial effects on antitumor activity and life prolongation of mice implanted with L1210leukemia. Additionally, key studies by Kingsbury et al. produced a number of analogues with substitutions at carbons 7, 9 and 10 that maintained good levels of activity. Also of importance were the initial reports of water insoluble analogues 9-aminoCPT (41) and 9-nitroCPT (rubitecan) (7), both of which were reported to possess levels of in vivo activity sufficient to merit clinical evaluation. Rubitecan (7) serves as a metabolic precursor to 9-aminoCPT and is currently in phase III clinical trials for the

treatment of pancreatic cancer. Among the derivatives reported by Kingsbury et al. for the first time were 9-((dimethylamino) methyl)-10-hydroxyCPT (topotecan) (3) and 10-hydroxy-9-((N-methylpiperazinyl)methyl)CPT (8), both of which displayed enhanced water solubility. These analogues illustrated successful strategies for substitution of the quinoline ring system and have been discussed in reviews. [46]

The demonstration that positions 7, 9, 10 and sometimes 11 of the quinoline ring system could be altered without diminishing topo I inhibitory activity and antineoplastic activity allowed researchers to examine a broader repertoire of derivatives. In recent years, efforts have been made to increase the water solubility, and conversely the lipophilicity, of CPT, to activate CPT as a prodrug and to produce conjugates with improved properties. A further effort has focused on manipulation of the lactone-carboxylate equilibrium (i.e., between 1 and 2), specifically in human plasma. The stability of the E ring lactone in human plasma can be affected by derivatization of the quinoline ring.

Considerable evidence supports the idea that small substituents at carbon 10 have little effect upon the ability of CPT to stabilize the enzyme–DNA binary complex, while larger groups can inhibit the ability of CPT to stabilize the covalent binary complex. For example, irinotecan (4), which has a large 4-(1-piperidino)-1-piperidinocarbamate group attached at carbon 10, does not stabilize the topo I–DNA binary complex, yet exhibits good clinical activity. Significant plasma concentrations of 7-ethyl-10-hydroxyCPT (SN-38) (5) in irinotecan treated mice bearing implanted L1210tumor cells prompted researchers to suggest that irinotecan functions as a prodrug. It has since been determined that irinotecan is indeed metabolized in vivo into SN-38. Human hepatic carboxylesterases have demonstrated high efficiencies in this transformation.[47] Further, irinotecan exhibited nearly 20-fold enhancement of lactone ring stability in human plasma relative to CPT.

In reflection of the clinical success of water soluble CPT derivatives topotecan and irinotecan, efforts to increase the water solubility of CPT have comprised a major

research focus. Some of the most successful water soluble derivatives have resulted from the use of aliphatic and aromatic amine substitutions at carbon 7 of 10,11-(ethylenedioxy) CPT. Initial reports of 10,11-(methylenedioxy)- 7-((N-methyl piperazino)methyl)CPT (lurtotecan) (9) highlighted the merit of this strategy and the compound has been in clinical trials for breast, colorectal and small cell lung cancers.[48] Further development led to the use of aromatic quaternary ammonium salts as water solubilizing functional groups. Derivatives such as 10,11-(ethylenedioxy)-7-pyraziniumylmethyl CPT chloride (10) have been reported to possess both superior water solubility and enhanced tumor growth suppression.[49] Another derivative that has received substantial attention is the water soluble exatecan (11). The potency of exatecan has been reported to be up to 28-fold greater than that of topotecan in the treatment of various human malignant cells; the compound is also a poor substrate for the protein BCRP which has been implicated in drug resistance.[50] Yet another study of Demir and Burke propose the preparation of camptothecin derivatives displaying cytotoxic activity and improved water-solubility for ease and efficiency of administration/delivery, by substitution of the 7-position of camptothecins or 12-position of homocamptothecins to form water-soluble compounds containing an aminoalkyl moiety. The aminoalkyl moiety may incorporate either an unbranched or branched alkyl chain. The homocamptothecin structure can be readily prepared starting from camptothecin according to a published method[51] or can be prepared following derivitazation at position 7.[52]

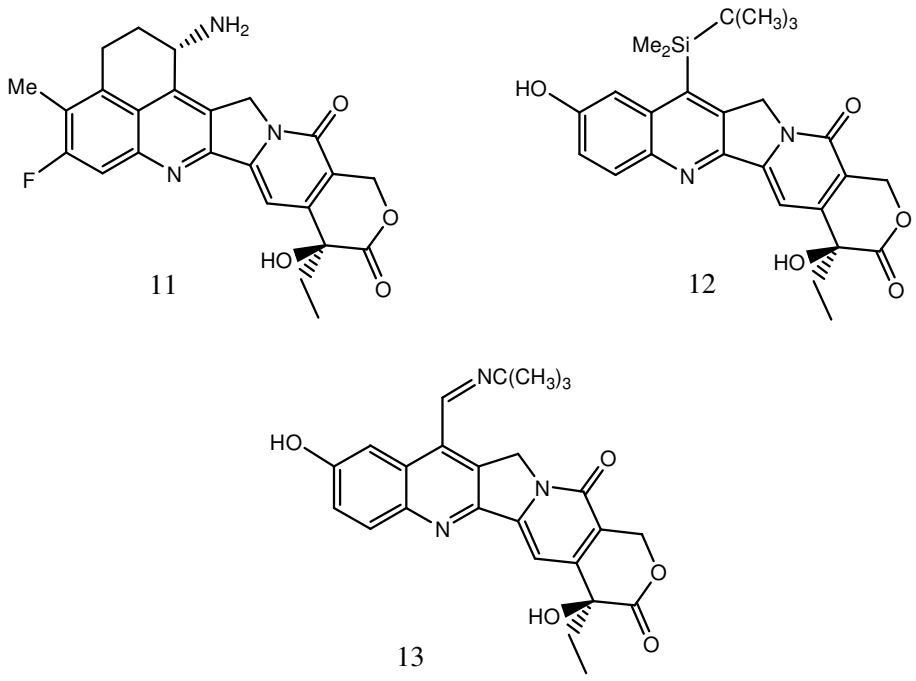


Figure 10: Quinoline ring modified camptothecins

Some workers have employed a different approach while endeavoring to establish novel CPT analogues. The need for an intact E-ring lactone in supporting the antitumor effects CPT was noted to be in conflict with the strong preference of human serum albumin for binding the carboxylate form of CPT, thus creating a potential obstacle in delivering the active form of the drug to tumor cells. Further, a quantitative structure activity relationship analysis of the National Cancer Institute drug database by Fan et al.[53] indicated that increased lipophilicity was highly advantageous and Burke et al. showed that the lactone was stabilized by passage through lipid bilayer. Hence, Curran et al. have prepared a number of silicon-containing CPT analogues (silatecans) that display increased lipophilicity.[54]

In addition to the preparation of novel lipophilic CPT analogues, lipid bilayer vesicles have been utilized successfully in the binding the lactone form of CPT, offering an additional approach to drug delivery. Moreover, the demonstration of the successful encapsulation of topotecan and irinotecan within such lipid carrier systems suggested

that the technique might well be applicable to a broad range of CPT derivatives. Silatecans, such as 7-(tert-butyldimethylsilyl)-10-hydroxyCPT (DB-67) (12), are more lipophilic than topotecan and are capable of elevated levels of liposomal core loading. Importantly, the silatecans have also demonstrated levels of cytotoxicity similar to that of CPT and elevated levels of in vivo activity; selected derivatives have entered into clinical trials.[55]

Zunino et al. have demonstrated that the use of appropriate substituents on the quinoline ring, including amino methyl, imino methyl and oxyimino methyl groups, are an alternative method by which the lipophilicity of CPT can be increased. Particularly successful were the oxyimino methyl derivatives substituted at carbon 7. Specifically, the alkylated oxyimino methyl derivatives with large lipophilic groups, such as t-Bu, produced highly active CPT analogues. Conversely, the addition of ionizable groups, such as carboxylic acids and amines, conferred unfavorable results. Like the silatecans, these novel C-7 substituted CPTs have demonstrated elevated lactone stability and shown superior antitumor efficacy in terms of tumor growth inhibition and rate of response. Among the most successful of these derivatives is the 7-(tert-butoxy iminomethyl)CPT (13), which caused complete tumor regression in mice bearing lung carcinoma LX-1.[56]

Few studies have focused on modification of the C and D rings of camptothecin (Figure 11). These have generally resulted in loss of activity as illustrated by homologated CPT derivative. The inability of these analogues to stabilize the covalent binary complex was attributed to the disruption of planarity. The diaza analogue (16), that should maintain quite similar shape and planarity relative to CPT, was found to be approximately 60-fold less efficient as a topo I inhibitor.[57] This finding presumably reflects the importance of the pyridone carbonyl of CPT in stabilizing the enzyme–DNA–CPT ternary complex.

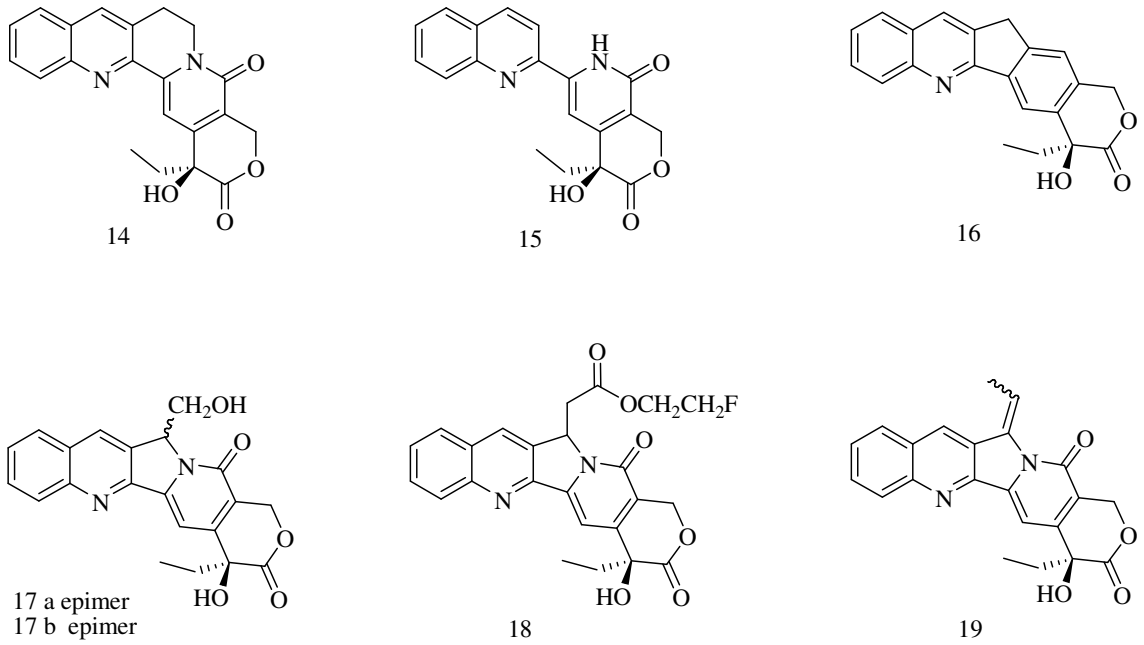


Figure 11: C/D ring modified camptothecin analogues

E ring substitutions have underscored the importance of certain structural features to the maintenance of the activity of CPT. Foremost is the importance of the α-hydroxylactone in supporting both efficient topoisomerase I inhibition and in vivo potency. Unfortunately, under physiological conditions, the presence of the α -OH group results in an equilibrium that favors the (inactive) open carboxylate over the (active) ring-closed lactone form. Hertzberg et al. replaced the lactone functionality with the more hydrolytically stable lactam, thereby reducing the tendency of the E ring to open. However, the resulting CPT lactam (20) (Figure 12) was ineffective as a topoisomerase I inhibitor. Other derivatives shown in the figure 12, including the carbinol lactam (21), the thiolactone (22) and imide (23) were also essentially inactive.

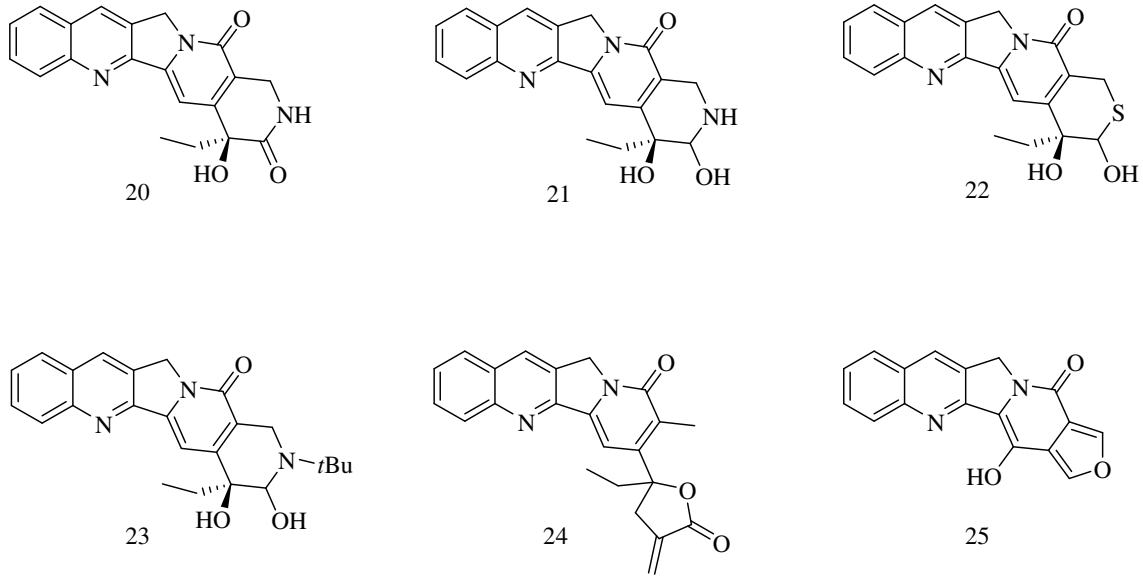


Figure 12: E ring modified camptothecin analogues

Hertzberg et al. also reported on the synthetically derived methylene lactone analogue (24) and a furanyl derivative (25), each of which possessed pronounced cytotoxicity (Figure 12). While these analogues were revealed to be effective DNA damaging agents, neither had activity as a topo I poison and their cytotoxicities were determined to be unrelated to topo I inhibition.

The stereochemistry at carbon 20 of CPT is apparently crucial, as the analogue having a 20(*R*)-OH group is inactive.[44] Further, the hydroxyl group itself is presumed to participate in one or more essential hydrogen bonding interactions. The ability to inhibit topo I mediated ligation and mediate cytotoxicity was retained by 20-aminoCPT (26) (Figure 13), an analogue capable of mimicking the hydrogen bond donor ability of the 20-OH group in CPT.[58] It has been suggested that an intramolecular hydrogen bond between the 20-hydroxyl group and the lactone carbonyl results in the activation of the lactone, furthering its susceptibility to nucleophilic attack.[59] At physiological pH, the amine functionality in analogue (26) would presumably be protonated and capable of the same intramolecular hydrogen bond donation as the 20-OH group normally present in CPT. Thus, the diminished activity of (26) demands reassessment of the role of the 20-

OH group. In this context, it may be noted that at physiological pH the 20-NH2 group would be incapable of acting as a hydrogen bond acceptor while a free OH could still participate in such a hydrogen bond. This could plausibly explain the decreased activity. The rapid rate of disassociation of (26) from the covalent binary complex, as demonstrated by NaCl induced disassociation experiments, is consistent with the diminished level of activity of (26), but does not provide a firm molecular basis for the reduced level of activity.[60]

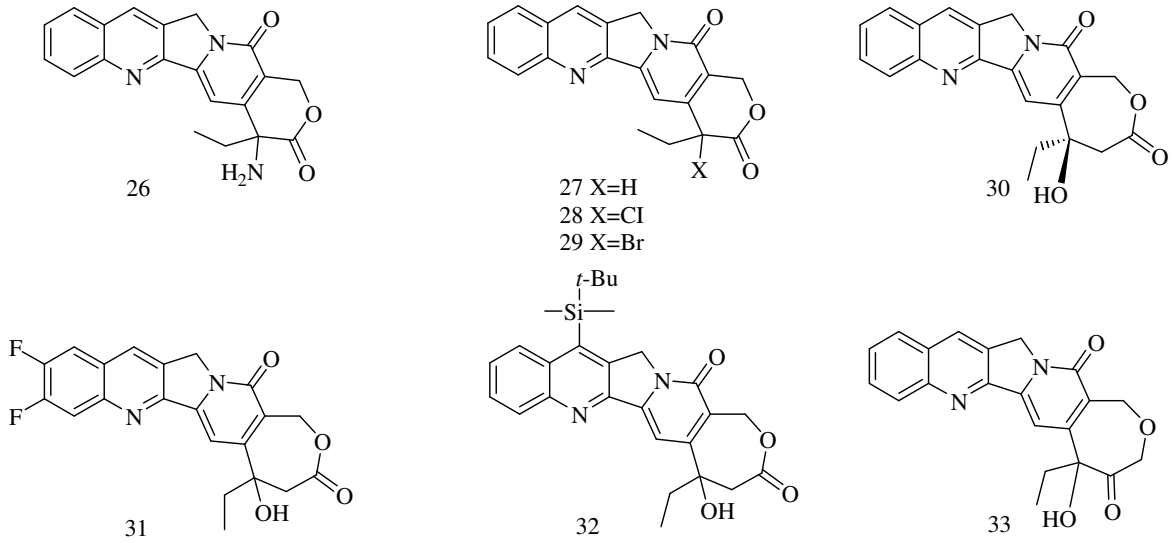


Figure 13: E ring modified camptothecin analogues

The 20-deoxy species (27) (Figure 13), which is incapable of hydrogen bonding in any fashion, was also shown to have significantly diminished topo I inhibition and no cytotoxicity, although it did inhibit a topoisomerase I induced rearrangement of DNA structure.[60] The substantial potencies of the homoCPT derivatives and the 20-chloro and 20-bromoCPTs (28 and 29) (Figure 13) argue that an intramolecular hydrogen bond to the lactone carbonyl is not essential.[60] However, it is noteworthy that the halogenated CPTs (28 and 29) are incapable of acting as hydrogen bond donors, yet capable of acting as hydrogen bond acceptors. Thus, the behaviors of 28 and 29 are altogether consistent with that of 20-amino CPT (26).

Replacement of the α-hydroxylactone moiety of CPT with a β-hydroxylactone was first reported by Lavergne et al.[42] HomoCPT (30) (Figure 13) was reported to exert potent inhibition of topo I and elevated levels of cytotoxicity. Further, the β-hydroxylactone was reportedly stable after 24 h at physiological pH. The demonstration that the seven membered β -hydroxylactone moiety did not diminish CPT activity has prompted the synthesis and evaluation of numerous derivatives.[42,61] Among the most promising of the homoCPT derivatives is 10,11-difluorohomoCPT (BN-80915) (31) which exhibited strong antiproliferative activity against numerous cell lines and is currently in phase I clinical trials.[62] Curran et al. have also introduced silicon into homoCPT derivatives, resulting in analogues having increased lipophilicity.[63] The 7-(tert-butyldimethylsilyl) homoCPT derivative (32) (Figure 13) is reported to display the highest level of lactone stability in both buffer systems and plasma. Interestingly, Du1441 (33) (Figure 13), an analogue similar to homoCPT, contains an α-hydroxyketo ether in place of the β-hydroxylactone, was inactive as a topo I inhibitor and in inhibiting cellular proliferation.[64] The inactivity of Du1441 again supported the thesis that an intact lactone ring is a structural feature essential for CPT function.

1.2.7 Conjugated analogues

The use of conjugates has emerged as a frequent strategy in efforts to optimize therapeutically beneficial properties of CPT, including lactone stability, solubility/lipophilicity, tumor cell recognition and sequence specificity of DNA damage (Figure 14). Two predominant methodologies have been utilized for the synthetic preparation of conjugates. The first relies on the utilization of the 20(S)-hydroxyl group as the site for conjugation. The second involves the exploitation of reactive functional groups, for example, amino, hydroxyl and carboxylic acid groups, on modified CPT analogues. Most of these groups have been part of the quinoline (A/B) ring system.

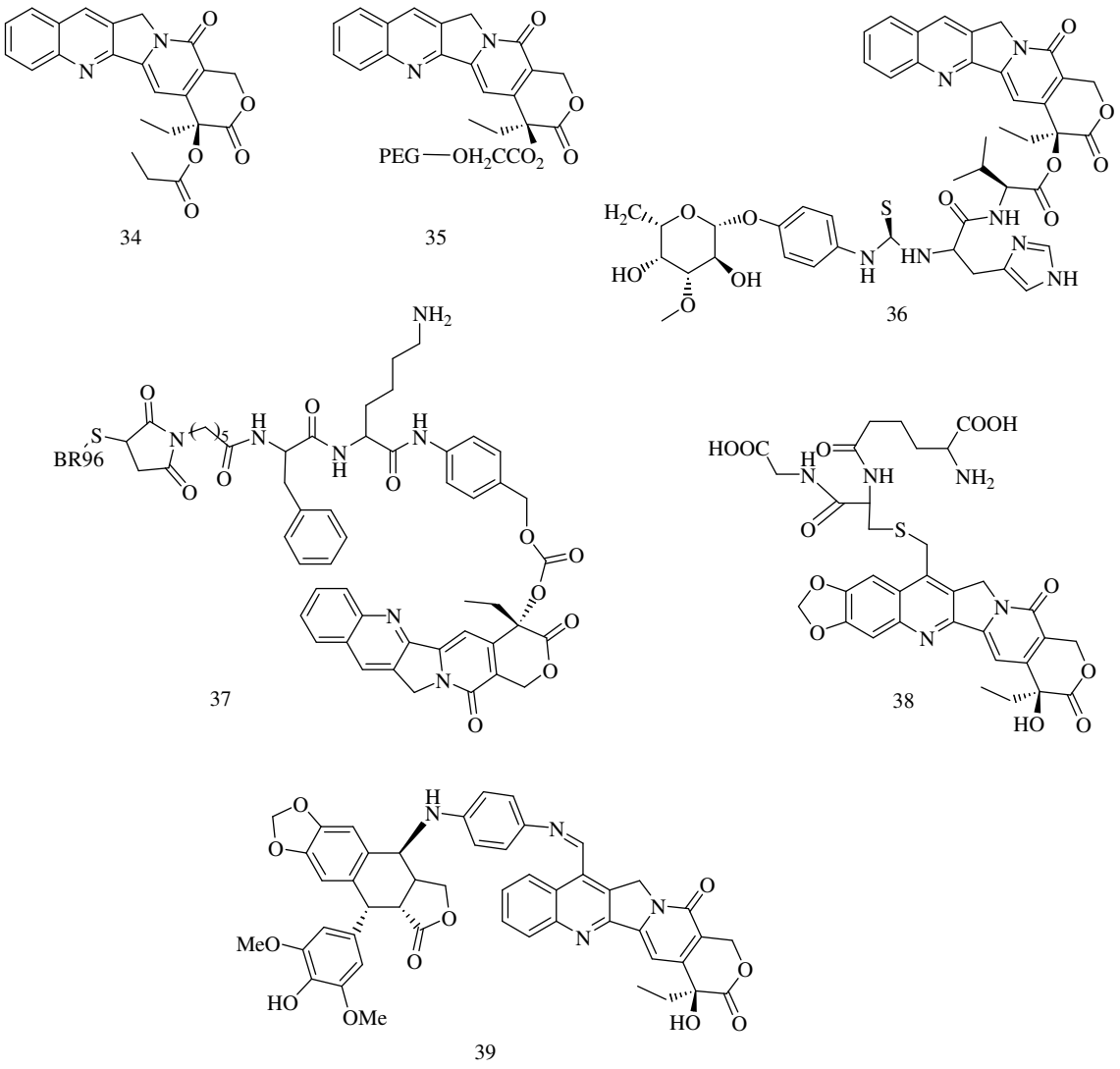


Figure 14: Conjugated camptothecin analogues

Perhaps the simplest of these derivatives have been obtained by esterification of the 20(S)-hydroxyl group. Giovanella et al. reported the synthesis and biological evaluation of a series of alkyl esters of varying size.[65] Specifically, the 20(S)-O-ethyl ester (34) was reported to possess reduced toxicities while demonstrating enhanced lactone stability and pronounced in vivo activities against human tumor xenografts in nude mice. Studies utilizing 20(S)-O-acyl esters resulted in CPT analogues with excellent in vitro cytotoxicity and antitumor activity equivalent to those of CPT.[66]

The utilization of 20(S)-O-amino acid analogues as prodrugs has also been studied. Interestingly, the isolation of 20(S)-O-β- glucopyranosyl CPT from Mostuea brunonis has been reported, suggesting that the 20(S)-hydroxyl group acts as a point of attachment for natural bioconjugates. A biosynthetic precursor of CPT glycosylated at the 20-position has also been reported. These studies have demonstrated the feasibility of using the 20(S)-hydroxyl group to modify CPT. The use of 20(S)-O-esters, amides, carbonates and carbamates with a variety of linkers have been employed in recent studies in the preparation of novel CPT conjugates. [67]

The antitumor activity, biodistribution and lactone stabilization have been reported for a series of polymeric conjugates of CPT, including poly(ethylene glycol) (PEG) and poly(N-(2-hydroxypropyl)methacrylamide) (PHPMA) conjugates, which were designed to be CPT delivery vehicles.[68] The polymeric PEG–CPT conjugate (35) was reported to produce improved levels of tumor regression in HT-29 xenografts and decreased toxicity relative to CPT. Fiebig et al. reported a 20(S)-O-linked glycoconjugate of CPT designed to improved cellular uptake of the drug.[69] Utilizing previously described fucoside residues to establish multivalent interactions between the drug–glycoconjuate and cell surfaces resulted in derivatives with excellent in vitro activity against HT29 tumor cells. This study also demonstrated the importance of the specific peptide linker in rendering such conjugates stable and biologically active.

In a recent study, Firestone et al. have reported the synthesis and initial biological profile of a CPT immunoconjugate designed to target tumor cells specifically.[70] Utilizing the tumor-recognizing antibody BR96 attached to CPT through a 20(S)-O-carbonate linker cleavable by cathespin B, the resulting immunoconjugate (37) was intended to eliminate the dose-limiting side effects while retaining the antitumor capabilities of the parent drug. The in vitro activity of the BR96 CPT conjugate was demonstrated to be superior to that of CPT alone.

The demonstration that substituents are well tolerated at carbons 7, 9 and 10of the quinoline ring (vide supra) has prompted the use of these positions for the

preparation of camptothecin conjugates. The successful conjugation of glucuronic acid to 9-amino CPT through an aromatic carbamate linker by Chern et al. exemplifies this approach.[71]

It has been reported that buthionine sulfoximine resulted in the cellular depletion of the ubiquitous antioxidant glutathione. It was further demonstrated that the depletion of glutathione potentiated CPT-induced cytotoxicity. Interestingly, a glutathione–CPT conjugate, 7-(gluta thionylmethyl)-10,11-methylenedioxyCPT (38) showed enhanced stability of the derived ternary complex .[72]

The observation that some CPT resistant cell lines concurrently developed resistance toward the topoisomerase II inhibitor etoposide, prompted Chang et al. to develop a CPT conjugate incorporating the 4b-amino- 40-O-demethyl-epipodo-phyllotoxin moiety of etoposide.[73] Two bioconjugates were synthesized differing only through the ortho (W2) and para (W1) phenylenediamine linkers and were shown to exert their cytotoxic effects primarily through topo I inhibition. Further, W1 (39) was demonstrated to be a more effective antineoplastic agent against human prostate cancer cells than either CPT or etoposide.

1.3 Photo removable compounds

With the increased understanding of the nature of photochemical processes and with sufficient instrumentation, preparative organic photochemistry has now been developed into an important branch of organic chemistry.[74] A newly developing field in which the principles of organic photochemical reactions are being advantageously exploited is in the design of photo removable protecting groups for the synthetic purposes. Sammes in his review on the application of photochemical reactions in natural product synthesis has made a brief mention of the two possible approaches for the design of protecting groups which may be removed by irradiation.[75] Specifically

removable blocking groups are of importance in many phases of organic synthesis, particularly in synthesis involving polyfunctional molecules that bear many functional groups to be protected. The protection is usually accomplished directly by converting the particular group into a derivative which is known to be stable under the experimental conditions to be used and from which the original group may be regenerated without affecting the synthesized molecule. The removal of these protecting groups involves, in general, either treatment with acid or base, catalytic or chemical reduction, or a combination of these methods. Sometimes the substrate may be sensitive to these conditions and may create problems. Therefore the use of protecting groups for which the regeneration of the protected function could be accomplished by photochemical means, avoiding the need for rigorous chemical treatment of the substrate, is an attractive alternative.

A prerequisite for a photochemically removable protecting group is a chromophore which is sensitive to light, but relatively stable to most of the wide variety of chemical reagents commonly encountered in the ground state manifold. The wavelength of the light to be used should be such that it will be absorbed only by the protecting group and will not affect other parts of the molecule. A good yield for the deprotecting step is mandatory for a protecting group. The photoreaction of the protecting chromophore should in no way affect the protected molecule and the photoproduct should be easily separable from the deprotected system. The lifetime of the excited state that is responsible for the deprotection reaction is of great significance. If the protected substrate has a long excited state lifetime before cleavage occurs the chances for undesirable quenching processes to reduce the efficiency of the cleavage reaction are greater. This fact would hinder the removal of the protecting group and also might labilise the quenching species. Thus, such energy dissipation processes might result in some undesired changes in the substrate. Therefore, it would seem a distinct advantage to have a protecting group with a short –lived excited state.

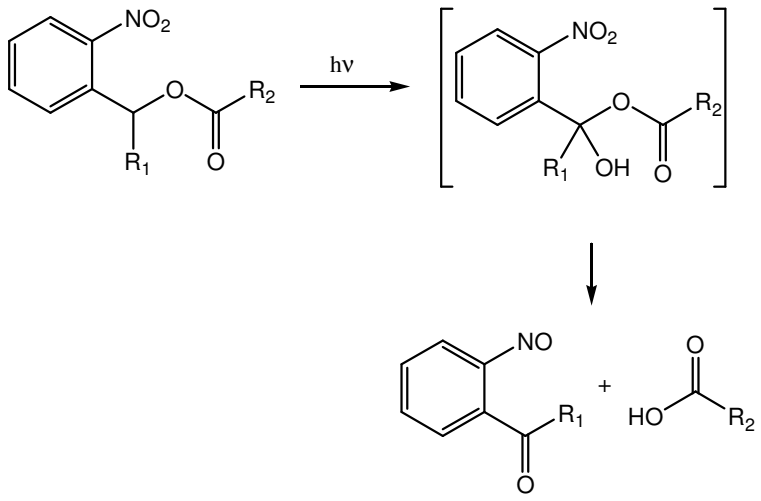
The first report of a successful development of a photo removable protecting group appears to be that of Barltrop and Schofield, who observed that benzyloxy-

carbonylglycine is readily converted to the free amino acid be irradiation with ultraviolet light.[76] Subsequently, a number of new photo removable protecting groups have been developed for various functional groups.

Today, photo removable protecting groups are being exploited in the synthesis of peptides, polysaccharides, and nucleotides. The most popular photo removable protecting groups can be listed under the subtitles below.

i. 2-Nitrobenzoyl and Related Compouns

The light-induced internal oxidation- reduction reactions of aromatic nitro compounds containing a carbon-hydrogen bond ortho to the nitro group have been the subject of much investigation.[77] A number of such intramolecular rearrangements are now known in which the nitro group is reduced to a nitroso group and an oxygen is inserted into the carbon-hydrogen bond located at the 2-position. The primary photochemical process in this case is established to be an intramolecular hydrogen abstraction by the excited nitro group. This is then followed by an electron redistribution to the aci-nitro form which rearranges to the nitroso derivative.[78] (Scheme 8)

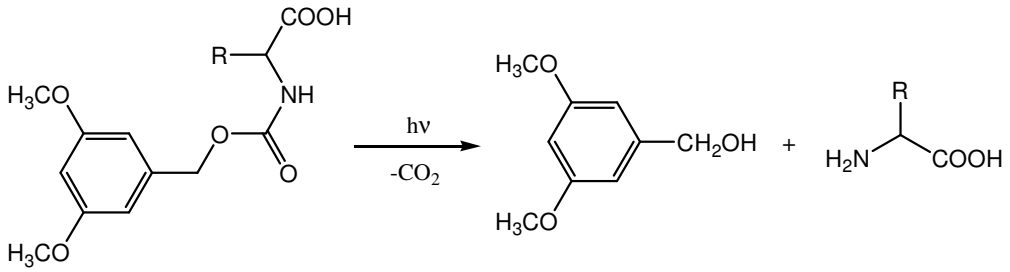


Scheme 8

This reaction has been utilized in the design of a number of related photosensitive protecting groups, all containing a carbon hydrogen bond in the ortho-position to the nitro group.

ii. Benzyloxycarbonyl and Related Groups

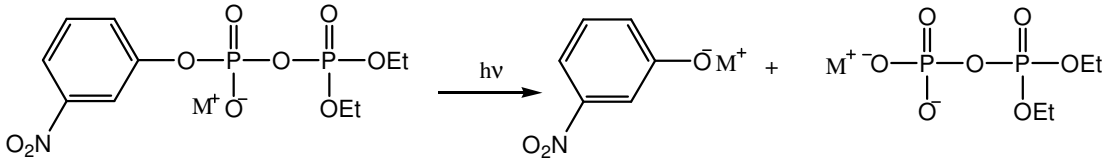
In the design of photosensitive benzyloxycarbonyl protecting groups, use is made of the photosolvolytic reactions resulting from the different reactivity of the excited aromatic compounds compared to that of their ground state. The benzyloxycarbonyl group, 3,5-dimethoxy benzyloxy carbonyl group and α,α-dimethyl-3,5-dimethoxy benzyloxy carbonyl group are three photosensitive protecting groups developed on the basis of this principle to give a urethane-type protection to the amino function in organic compounds. These protecting groups can be removed by irradiation with light of appropriate wavelength under conditions which do not affect the system which is being protected. (Scheme 9)[76,79]



Scheme 9

iii. 3-Nitrophenyl and Related Groups

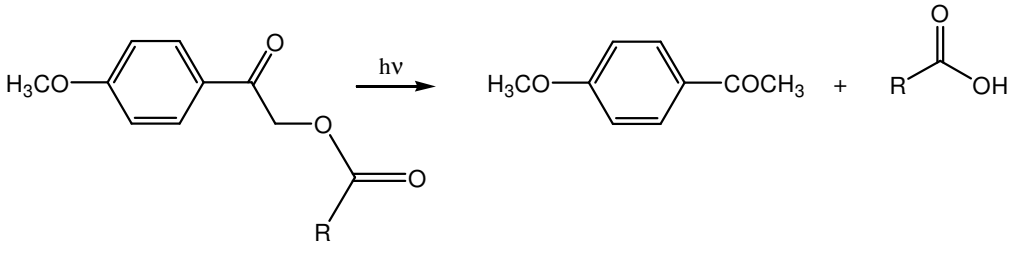
As in the case of 3,5-dimethoxy-substituted aromatic systems, the enhanced reactivity of the excited meta-substituted aromatic compound compared to that of the ground states is the driving factor for the photocleavability of the 3-nitrophenyl and the related groups (Scheme 10). [80]



Scheme 10

iv. Phenacyl Groups

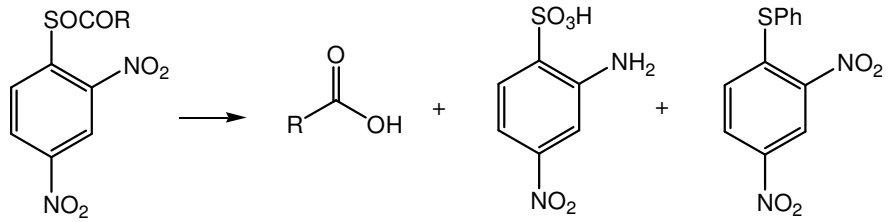
Because of the interaction between the electrons of the carbonyl group and the phenyl ring, the phenacyl group has low-lying excited states. Such interactions make phenacyl esters photolytically cleavable. (Scheme 11) This photolytic cleavage is made use of in the design of phenacyl protecting groups for the carboxylic function.[81]



Scheme 11

v. 2,4-Dinitrobenzenesulfenyl Groups

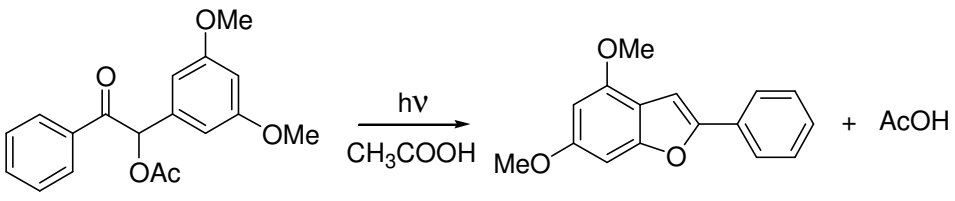
The ease with which 2,4-dinitrobenzenesulfenyl esters can be photolysed to liberate the corresponding carboxylic acids in good yields suggested its use as a potential photosensitive protecting chromophore for the carboxylic function.[85] The protecting group photoproducts, in this case, are 2-amino-4-nitrobenzenesulfonic acid, 2,2',4,4'-tetranitrodiphenyldisulfide and 2,4-dinitrodiphenyl sulfide. (Scheme 12)



Scheme 12

vi. 3,5-Dimethoxybenzoinyl Groups

One class of protecting groups that have seen less use is the substituted benzoins initially reported by Scheenan et al.[83] Of particular interest are the substituted alkoxybenzoins which have quite remarkable photocleavage properties. An example is the 3',5'-dimethoxybenzoin protecting group which undergoes photoinitiated cyclization and cleavage as shown in scheme with a rate constant estimated to be greater than 10^{10} sec^{-1} and a quantum efficiency of 0.64, where R_1 is acetyl. (Scheme 13)



Scheme 13

Such a protecting group is of considerable interest, not only in synthesis but also for the study of rapid enzyme kinetics and several recent papers have described the use of the 3',5'-dimethoxybenzoin as a protecting group in oligonucleotide synthesis and for caged substrates. Advantages of the 3',5'-dimethoxybenzoin versus substituted nitrobenzyl protecting groups are the rapid release rate, high quantum yield and the easily detectable nonreactive benzofuran photo product.

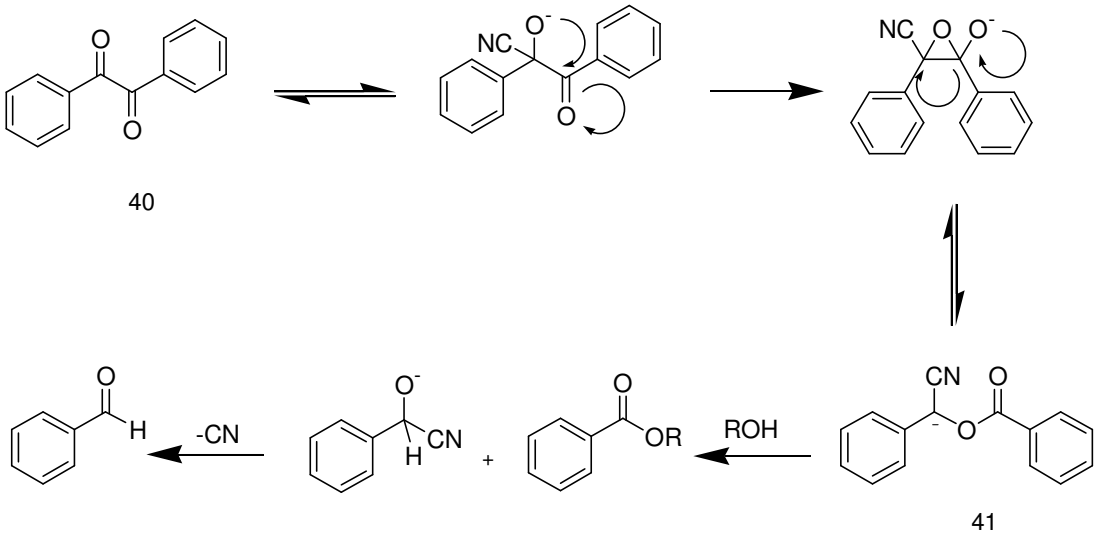
The 3',5'-dimethoxy protecting group seems to have two distinct disadvantages in application. First due to the remarkable photocleavage properties of the 3',5'-dimethoxybenzoin protecting group, photolysis can occur in standard laboratory light and samples must be kept in complete darkness. Secondly while considerable effort has gone in the synthesis of acyloins, many of these methods have proven to be inefficient for a number of targeted acyloins, in particular 3',5'- dimethoxybenzoin. Thus, synthesis of such compounds -which can be defined as unsymmetrical benzoin- is a matter of research.

1.4 Benzoin Chemistry: Synthesis of unsymmetrical benzoins

Unsymmetrical benzoins can be synthesized efficiently from trimethylsilyl (TMS) ethers of aryl cyanohydrins, either by addition of a Grignard reagent to the nitrile or by metallation followed by addition of the cyanohydrin carbanion to an aromatic aldehyde.[84] These methods have certain drawbacks such as use of air sensitive reagents and protection-deprotection steps.[85] Recently an excellent method was disclosed in which acylsilanes produce triethylsilyl ether of cyanohydrin in the cyanide ion-catalyzed reaction.[86] This intermediate reacts with aldehydes to afford the corresponding silyl protected benzoins in high yields. However, the syntheses of acylsilanes are generally accomplished via the corresponding dithianes that also requires the use of air sensitive reagents and some laborious protection-deprotection steps.[87] Although C-C forming enzymes have been shown to provide unsymmetrical benzoins in enantiomerically pure

form, its applicability is limited to the use of only a few aldehydes; and enzymes mediating this reaction are not readily available. Some other methods have also been reported to provide less stable unsymmetrical benzoins, but the versatility of the reaction is limited by low yields (30-50%) and side products.[85]

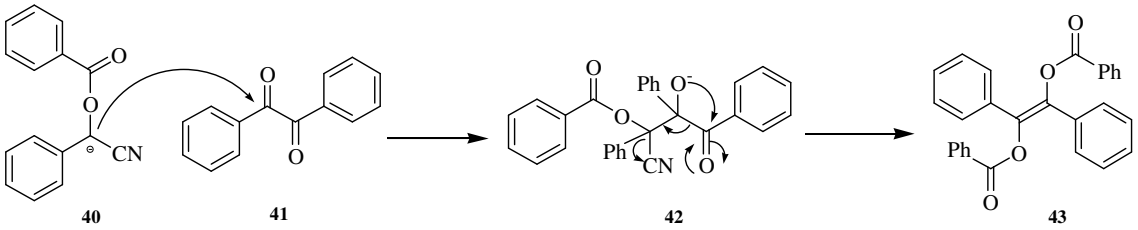
In 1923, Dakin and Harington showed that the cyanide ion catalyzes the cleavage of benzil to benzaldehyde and the ester of benzoic acid.[88] Later, the mechanism and kinetics of the reaction were investigated by Kwart and Baevsky.[89] They showed that the cyanide ion was able to cleave a series of benzils depending on the substitution on the aromatic rings, and proposed a mechanism, given in the scheme 14.



Scheme 14

The formation of derivatives of (41) from O-benzoyl cyanohydrin under highly basic conditions was reported.[90] The reaction of derivatives of (41) with aromatic aldehydes has been shown to form corresponding benzoin benzoates in low to moderate yields. These low yields are possibly related to both the poor reaction conditions for generating (40) and the stability of the products under the given conditions.

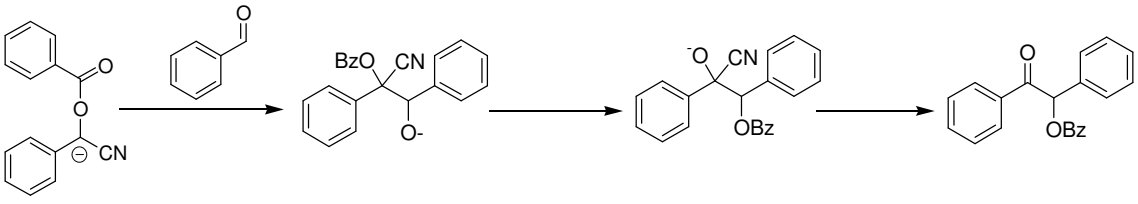
After Kwart and Baevsky proposed the intermediacy of (41) in the cyanide anion catalyzed cleavage of benzil, Trisler and Frye showed that (41) derived from benzil reacts again with benzil (40) in aprotic solvent DMSO, where (41) is highly nucleophilic, to form *trans-α,α'*-stilbendiol dibenzoate (43) through (42) (scheme 2).[91] This work showed that (40) is a potent nucleophile and can react with a potent electrophile in the medium. (Scheme 15)



40 41 42 43

Scheme 15

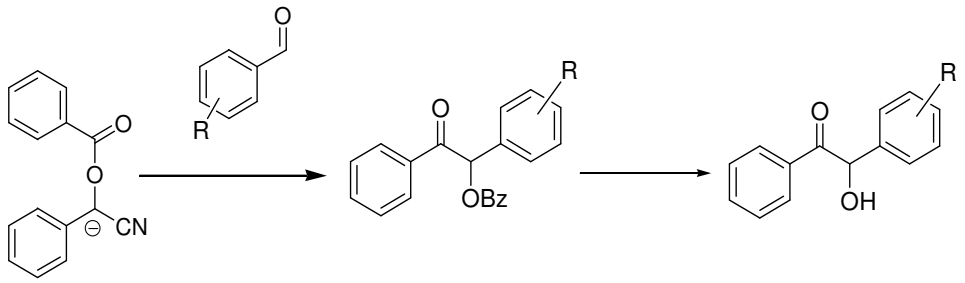
Later, Kuebrich and Schowen, in a study investigating the nature of α-hydroxycarbanions that are reactive intermediates in benzoin reactions, used the benzil and cyanide in DMF to generate the intermediate (40) and examined its reaction with benzaldehyde and furfural.[90] Although (40) could be generated efficiently under aprotic conditions, methods of utilizing it have not been developed and well understood; otherwise it would be quite useful for cross benzoin synthesis in both free and protected form. (Scheme 16)



40 44

Scheme 16

Lastly Demir and Reis[92] have shown that the cyanide ion catalyzed cleavage of aromatic α-diketones can be used for the generation of various masked acyl intermediates. These intermediates were reacted with various aromatic aldehydes to form the corresponding esters of unsymmetrical benzoins in high yields and can be converted to corresponding benzoins upon hydrolysis. A variety of different benzoin derivatives can be synthesized in this way in addition benzoate ester products may be used as photoprotective groups. (Scheme 17)



Scheme 17

1.5 Aim of the Work

In this study modification of the CPT stucture in order to improve its stability and solubility without reducing its activity was investigated. There are many attemps for the synthesis of new functionally improved molecules in which functionalization of CPT at various sites were shown to be beneficial from different aspects. Our approach consists of two main title, modification from 20-OH position and modification from C-7 position. In both cases we suggested to overcome the stability and solubility problems of camptothecin and also we provided a new strategy for a targetting release of camptothecin.

The first part of the study involves the modification from 20-OH position. In this part of the study we developed a new strategy for targeting release of camptothecin in

which the drug was hidden with a photoremovable group like the 'Trojan Horse' case. The 20-hydroxyl group of the camptothecin is the most essential –by means of activity- and the most sensitive –can be hydrolysed easily in physiological conditions- part of the molecule. Thus, we first aimed the protection of the 20-hydroxyl group so-called the "Achhile's heel" of the molecule. For the controlled release purposes we decided to use photoremovable benzoin derivatives which are known to be very effective in photorelease reactions but are disadvantageous since it is difficult to synthesize unsymmetrical benzoins. However, in our group a new method was developed for the synthesis of unsymmetrical benzoins and the easiness of this method gave rise to the development of new unsymmetrical benzoin derivatives substituted with some representative molecules. The retrosynthetic pathway of the new camptothecin derivatives is given below (Figure 15).

In the other part of the study modification of C-7 position was concerned. Early studies, have indicated the importance of lipophilic groups in position 7 for potent CPT activity. The 7-amine group substituted CPT and 7-silyl CPT derivatives found to show potencies of antitumor activity comparable to or better than CPT itself. Substitution of C-7 with silyl amino functions to provide CPT stability was involved in the other part of the study. Different types of reactions with different pathways were applied inorder to achieve the goal.

Besides the studies involving the synthesis of substituted CPT derivatives, the idea of CPT connected with cisplatin was developed. Cisplatin is one of the most effective anticancer agents but its clinical use is limited due to its adverse effects that are observed in patients. Connection of cisplatin with CPT may overcome the existing application problems of both drugs and may exhibit a dual effect on the treatment of some cancer types.

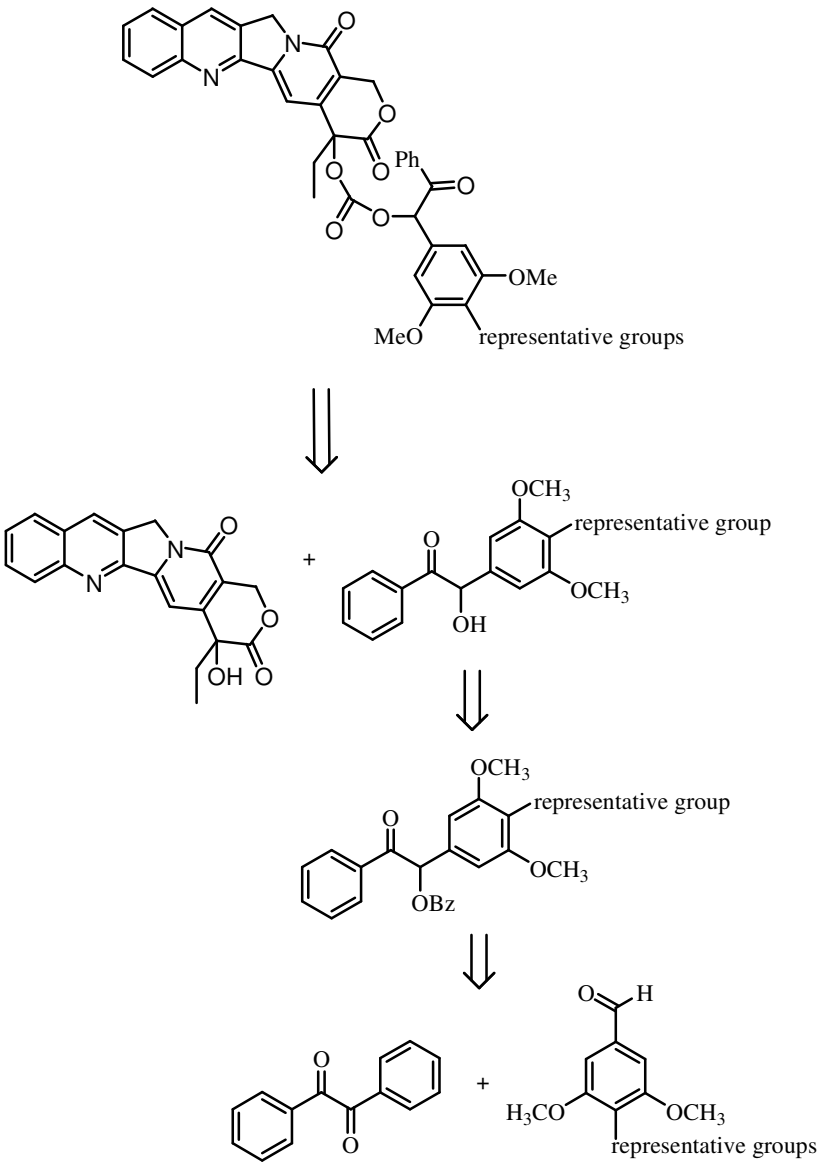
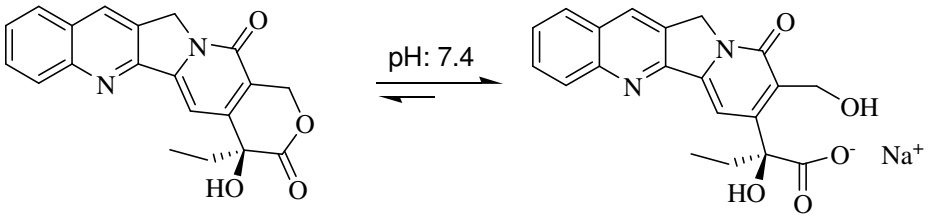


Figure 15: Aim of the work

CHAPTER 2

RESULTS AND DISCUSSION

CPT is clinically problematic for two reasons. Firstly, CPT is insoluble in all organic solvents except dimethylsulfoxide in which it has high solubility. Secondly, the drug is highly pH sensitive, the active lactone moiety of the drug can rapidly undergo reversible hydrolysis and be converted to the inactive ring open form under physiological conditions (Scheme 18).[1,2] Clinical trials carried out using the water soluble sodium salt, resulting from the hydrolysis of CPT by sodium hydroxide, were discontinued because of severe and unpredictable toxicity (Scheme 18). [3]



1 2

Scheme 18

2.1 20-OH substituted CPT derivatives

In terms of hydrolysis, each of the clinically relevant camptothecins contain an α–hydroxy-δ-lactone pharmacophore; at pH 7 and above, this functionality is highly

reactive and readily converts to the "ring opened" carboxylate form. Unfortunately, the carboxylate form of the camptothecin agent is inactive. Thus, as a result of the labile α–hydroxy-δ-lactone pharmacophore, camptothecins exist in an equilibrium consisting of two distinct drug species: (1) the biologically active lactone form in which the lactone ring is closed; and (2) a biologically inactive carboxylate form generated upon the hydrolysis of the lactone ring of the parent drug.[93]

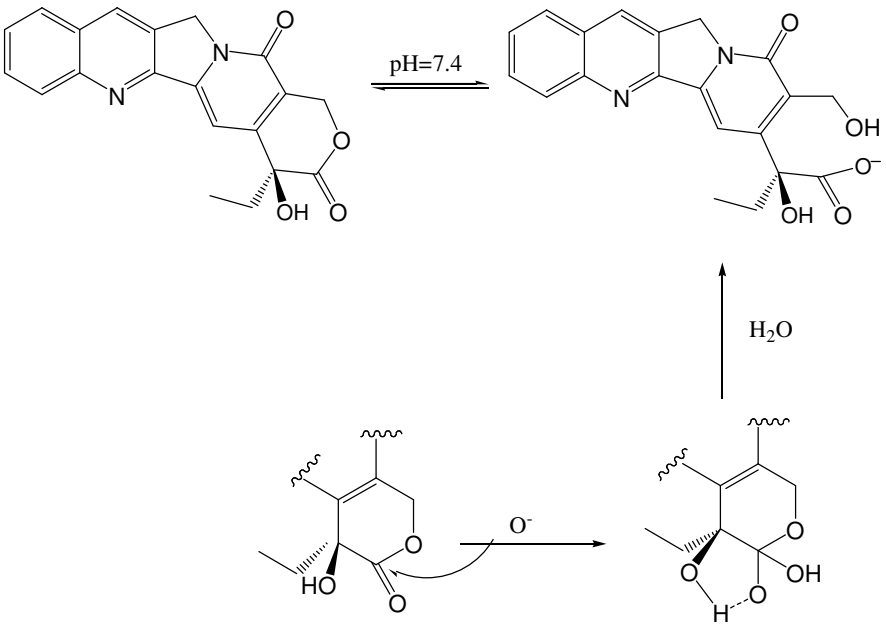
Unfortunately, the hydrolysis problem with CPT and many analogues (e.g., 9 aminocamptothecin, 9-nitrocamptothecin) is exacerbated in human blood. In human blood and tissues, the CPT equilibrium of active lactone form versus inactive carboxylate form can be greatly affected by the presence of human serum albumin (HSA). Time-resolved fluorescence spectroscopic measurements taken on the intensely fluorescent CPT lactone and CPT carboxylate species have yielded direct information on the differential nature of these interactions with HSA. The lactone form of CPT binds to HSA with moderate affinity, yet the carboxylate form of CPT binds tightly to HSA, displaying a 150-fold enhancement in its affinity for this highly abundant serum protein. Thus, when the lactone form of CPT is added to a solution containing HSA, the preferential binding of the carboxylate form to HSA drives the chemical equilibrium to the right, resulting in the lactone ring hydrolyzing more rapidly and completely than when CPT is in an aqueous solution without HSA. These dynamic processes present a major hurdle to achieving successful chemotherapy of a cancerous disease state.[94]

For example, the carboxylate forms of CPT, 9-aminocamptothecin and 9-nitrocamptothecin, exhibit a very high and species-specific affinity for human serum albumin. Perhaps as a result of these species-specific interactions, the clinical translation of the impressive biological activities observed in murine models has been very difficult to achieve. The interspecies variations in albumin binding noted for these camptothecins may in part explain why 9-aminocamptothecin (9-AC) was highly effective against human cancer in xenograft models but has performed poorly in humans trials. In addition to modulating human blood stability, the presence of physiologically relevant concentrations of HSA can greatly attenuate (by orders of magnitudes) the anticancer

activities (IC50 values) of these agents. In humans it appears that protein binding interactions make it difficult to achieve therapeutically effective unbound lactone levels of these agents, particularly when *continuous* exposures (for tumors cells to cycle through S-phase) of the active lactone form are requisite for efficacy purposes is considered.[94]

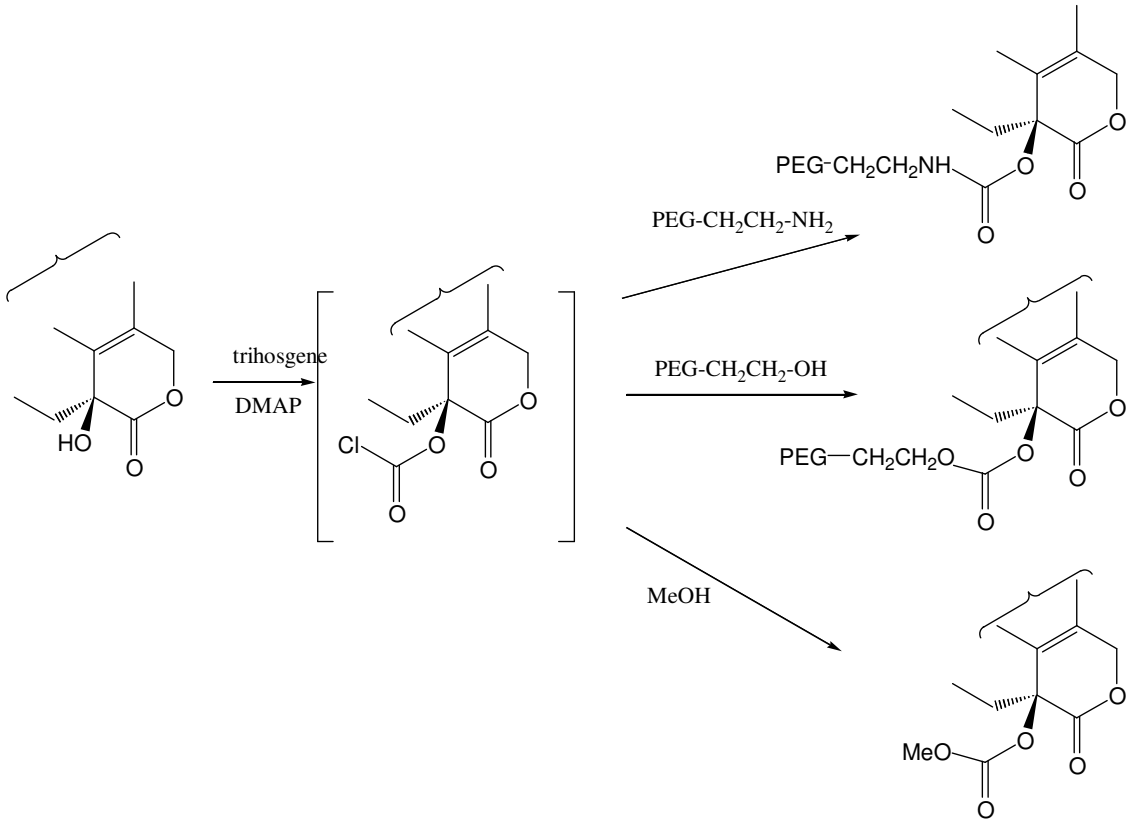
Many attemps have been made to design potent camptothecins displaying improved stabilities in human blood. As it was described above, prolonging the biological life span of an active drug by protecting the lactone moiety of the molecule is important for drug development. An attempt made by Wall et.al. was to convert some synthetic (RS)-camptothecin derivatives into the corresponding water-soluble glycinate hydrochloric salts. These glycinate hydrochloric salts were tested in vivo against L1210 cells and showed less potency when compared with camptothecin. Whether these glycinates could prolong the biological life of the active drug was not addressed.

Cao et al.[95] proposed the intact lactone ring would be better protected if camptothecins were transformed into the corresponding water-soluble alkyl esters. This proposal is supported by Fassberg and Stella[96] who have postulated that CPT lactone ring opening arising from nucleophilic attack at the acyl carbon, probably involves the 20-OH group in one of the proton-transfer steps or in stabilizing the transition state perhaps via a strong intramolecular H-bond as shown in scheme 19.



Scheme 19

Zhao et al,[97] have shown that application of a polyethylene glycol (PEG) prodrug strategy carried out by conjugation of CPT directly to PEG diacid results in a higly water soluble delivery system for CPT parental administration. They also presented HPLC experimental data to support their premise that esterification of the 20-OH of CPT stabilized the lactone ring, a phenomenon previously overlooked (Scheme 20).



Scheme 20

In the present study, three main goals were objected to be achieved; demonstration of improved stability, improved solubility and targeted release of new camptothecin derivatives. The main objective of the study addressing these goals is the conjugation of photolabile protecting groups to CPT on 20-OH position. The designed molecule is shown in the figure 16 below.

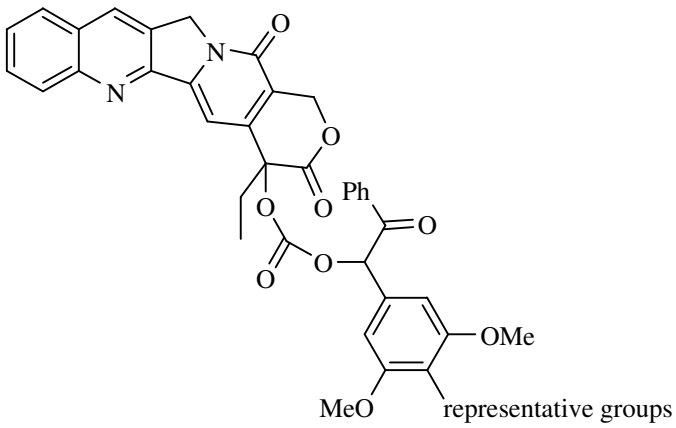


Figure 16: New camptothecin derivatives

These derivatives are supposed to be prodrugs of CPT that can undergo C-O bond cleavage and decarboxylation to release free CPT under UV radiation (~350 nm). So these can be used as a controlled release system for CPT and benzoin chromophores can be further modified for targeting.

Classical prodrug design often represents a nonspecific chemical approach to mask undesirable drug properties such as limited bioavailability, lack of site specificity, and chemical instability. On the other hand, targeted prodrug design represents a new strategy for directed and efficient drug delivery. Particularly, targeting the prodrugs to a specific enzyme or a specific membrane transporter, or both, has potential as a selective drug delivery system in cancer chemotherapy. Site-selective targeting with prodrugs can be further enhanced by the simultaneous use of gene delivery to express the requisite enzymes or transporters.[98] Our study thus comprises the construction of the "Trojan Horse", masking the drug with the photoremovable benzoin derivatives substituted with the representative groups serving as a courier. The molecule is supposed to travel with the guidance of these molecules, placed on the tumor and release the drug under UV radiation.

2.1.1 Synthesis of Photoremovable Unsymmetrical Benzoins

First reported by Barltrop and Schofield in 1962, photolabile protecting groups have found numerous applications in biology and chemistry in the past decade. The protecting groups (also known as "caging" groups) can render a bioactive compound inactive until they are removed by photolysis, thus releasing the compound rapidly. These properties of caged compounds, the inertness of a precursor, and the rapid release of a bioactive compound after an equilibrium with another reactant has been achieved make the photolabile precursors particularly useful for many controlled release purposes.

Banerjee et al.[99] reported the following criteria of a caged compound must satisfy for the usage in biological systems or controlled release purposes.

(1) The compound must be stable.

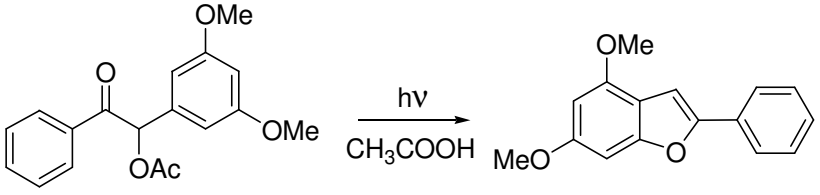
(2) The compound should be photolyzable at long wavelengths to avoid cell damage (visible region preferred, wide infra).

(3) The quantum yield of the photorelease should be sufficiently high that the bioactive compound is released in quantities adequate for the proposed studies.

(4) The release of the bioactive molecule should occur in acceptable time.

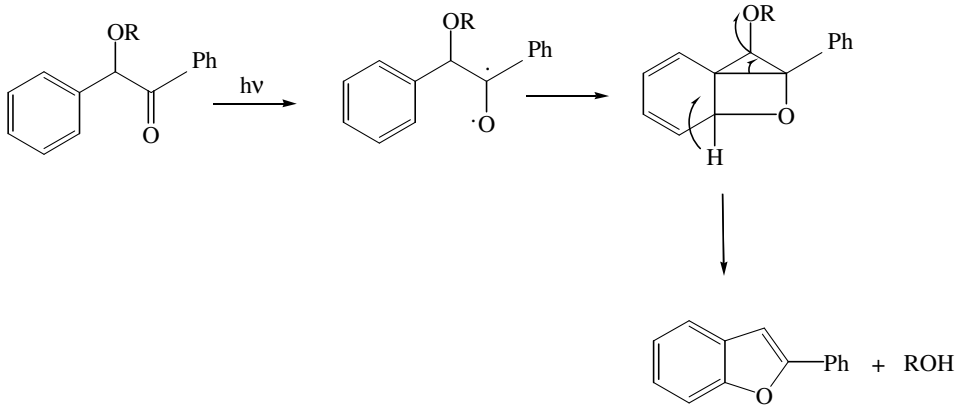
(5) The caged compound and the photolysis byproducts must be biologically inactive.

In recent years there has been considerable interest in photolabile derivatives of benzoins, and in particular 3'-5'-dimethoxybenzoin. In 1971 Sheehan et. al. showed that esters of the latter compound undergo efficient and very clean photolysis under near UV illumination. (Scheme 21)



Scheme 21

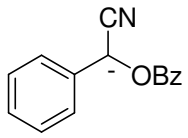
Sheenan et.al [83] proposed that absorption and emission data for these benzoin esters indicate that in all cases the lowest excited singlet and triplet states are associated with the benzoyl carbonyl and may be considered n-π * in the solvent systems used in this work. The observation that the cyclization is enhanced by the presence of an appropiately situated electron-donating group is consistent with the electrophilic nature of the n- π * carbony state. On the basis of these considerations, the propensity of the n-π * excited carbonyl to form oxetanes, and the heterolytic requirement imposed on the ester cleavage step, the following mechanism for benzofuran formation is favored. The proposed mechanism of the conversion of benzoin derivatives to benzofuran derivatives is shown in scheme 22.



Scheme 22

The first objective of our study was to synthesize new unsymmetrical benzoin derivatives which have comparable photo release properties with 3'-5'-dimethoxybenzoin.

The synthesis of desired unsymmetrical benzoins was achieved by cyanide ion catalyzed cleavage of benzils which was reported by Demir and Reis.[85] According to their report, the cyanide ion catalyzed cleavage of aromatic diketones can be used for the generation of various masked acyl intermediates (40) that is shown in the figure below.

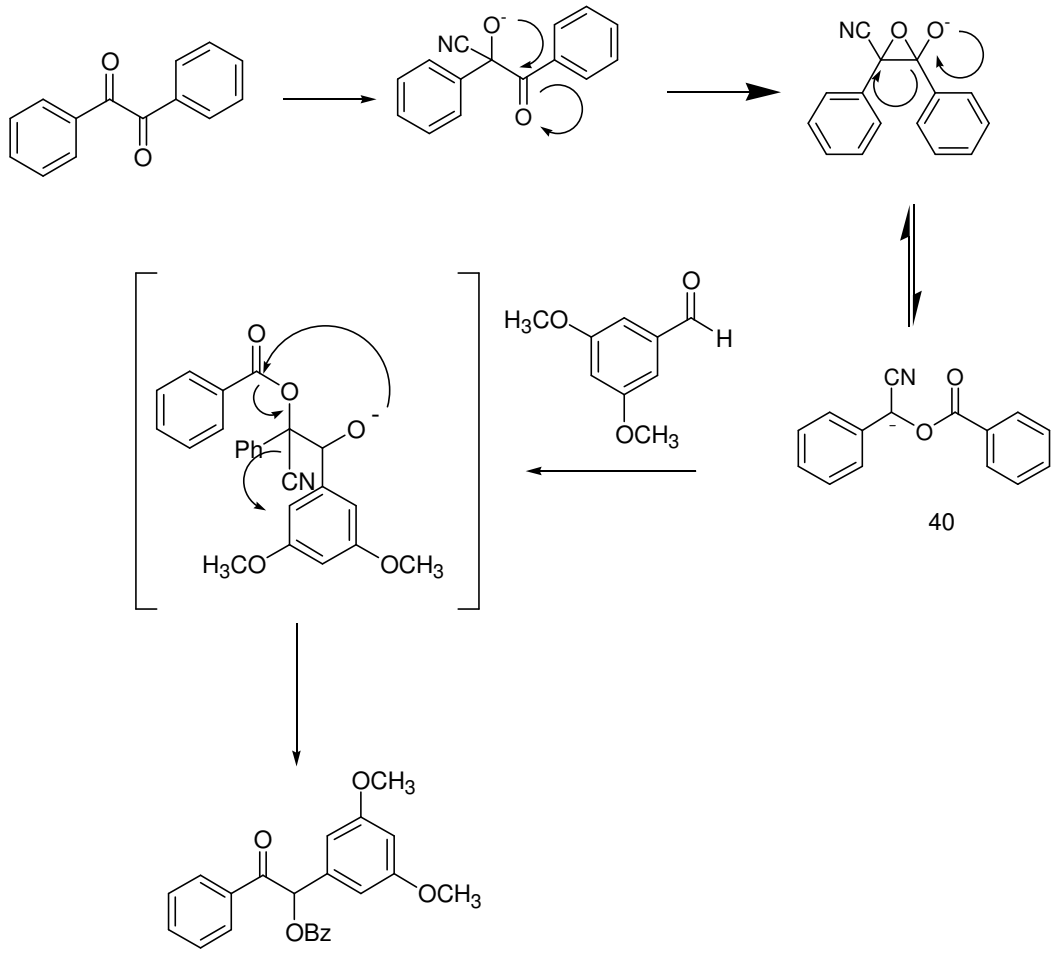


40

Figure 17: Acyl intermediate

These intermediates were shown to be able to react with various aromatic aldehydes to form corresponding esters of unsymmetrical benzoins in high yields. In this study a variety of different benzoin derivatives were synthesized by this way. Their method is applicable for our synthesis since it does not require the handling of air sensitive reagents and protecting groups.

The proposed mechanism for the reaction of benzil with 3,5-dimethoxy benzaldehyde under the catalysis of KCN was shown in scheme 23.



Scheme 23

The 3',5'-dimethoxy benzoyl benzoin was synthesized as the initial study by reacting benzil with 3,5-dimethoxy benzaldehyde in the presence of KCN in DMF and the desired molecule was synthesized as it was shown in the above scheme. (Scheme 23) This molecule is very important for us since it represents a reference for the photolysis experiments.

The Rayonet Reactor is a commonly used instrument in the photolysis reactions. The instrument is equipped with the UV lights of selected wavelength range and optionally a merry go round set up is used to provide homogeneous exposion of the light. A standart rayonet reactor is shown in figure 18.

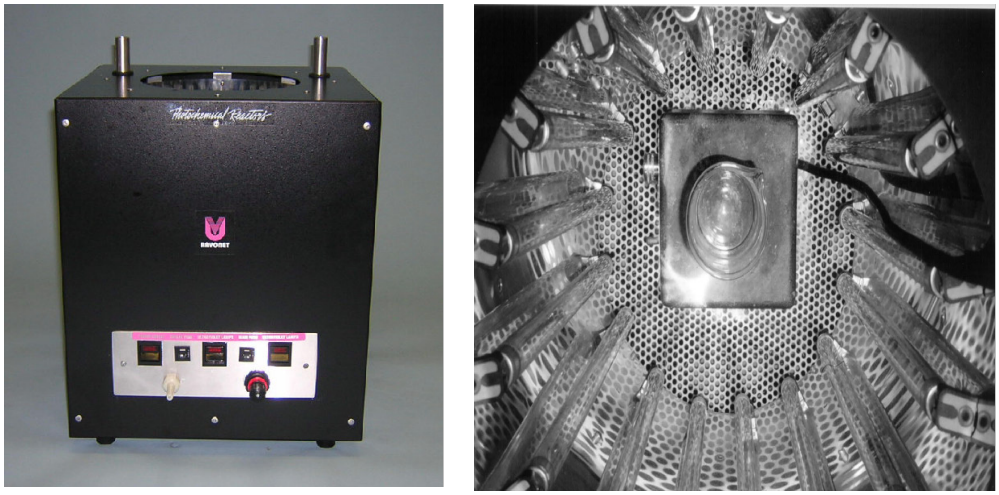
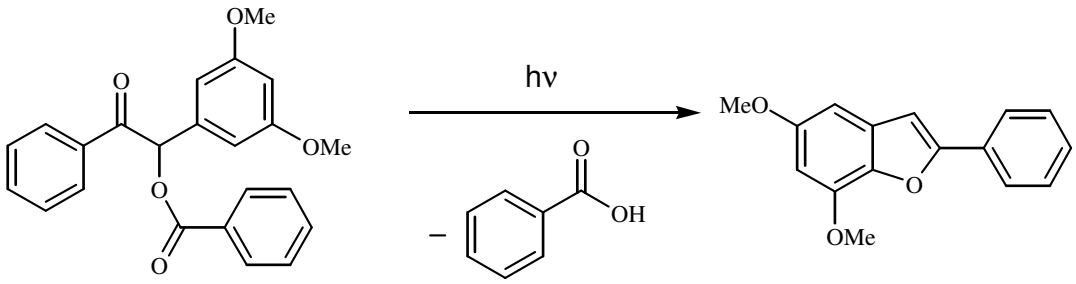


Figure 18: Outside and Inside view of a Rayonet Reactor equipped with a merry go round apparatus

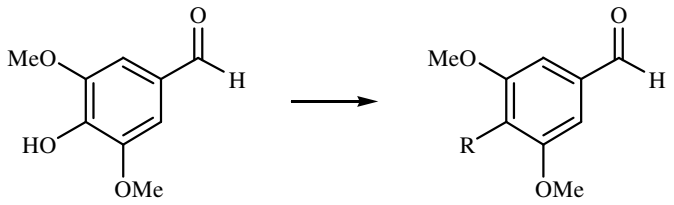
The photorelease experiments of this study are done in a hand-made Rayonet reactor that is equipped with four UV lights of 350 nm and a cooling fan. The 3',5'-dimethoxy benzoyl benzoin solution placed in a quartz tube is then exposed to light in our rayonet reactor. The reaction was monitored with TLC and the conversion of the benzoyl benzoin to the corresponding benzofuran derivative was achieved in almost quantitative yield in approximately 30 minutes (scheme 24).



45 46

Scheme 24

As the second step of the study, new benzoin derivatives substituted with representative groups were synthesized starting from suitable aldehydes. Firstly the aldehydes with the representative groups on the 4th position were synthesized using 3,5-dimethoxy-4-hydroxy benzaldehyde as precursor. The reaction scheme and the substituents were given in the below scheme 24. The substituents on the benzoins are all representing a special group or a purpose.



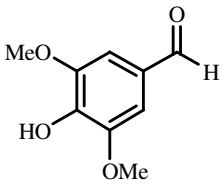
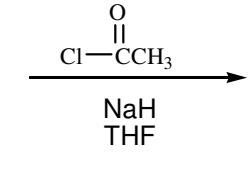
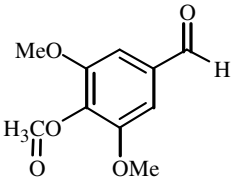
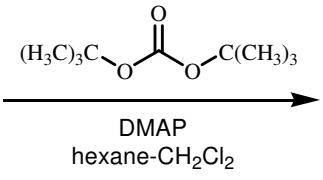
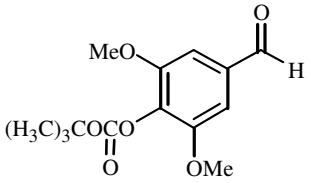
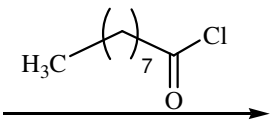
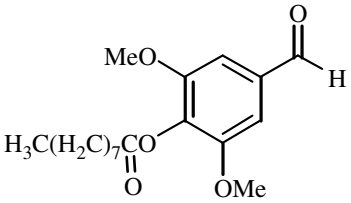
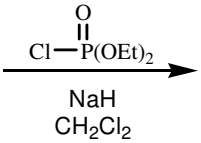
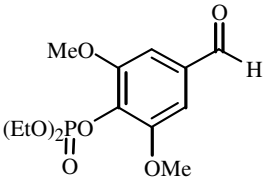
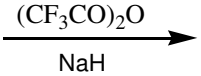
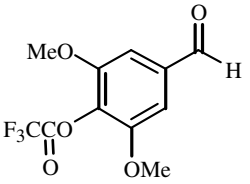
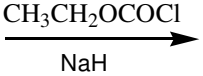
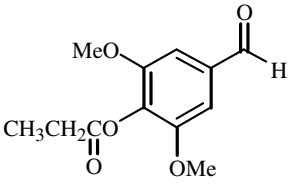
47 48

R	representative for
49: -H	as a reference molecule
47: -OH	can be functionalized easily
50:-OMe	alkyl segment
48a: -OAc	esterification
48b: -OCOOC$(CH_3)_3$	can be hydrolyzed easily
48c: -OCO$(CH_2)_8CH_3$	long chain
48d: -OPO$(OEt)_2$	DNA segment

Scheme 25

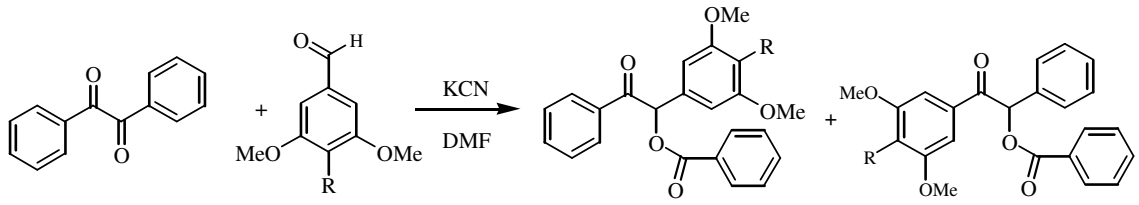
The syntheses of aldehydes were carried out by simple reactions in which known procedures were followed. The aldehydes 47, 49, 50 were commercially available aldehydes and the others were synthesized from 47 in the presence of NaH in anhydrous THF. In the case of 48b a different process were applied. The experimental conditions and the chemical yields were given in the table below. In all cases the products were purified by column chromatography and assigned by ^{1}H NMR spectroscopy. (Table I)

Table 1

		product	% yield
 47	 NaH THF	 48-a	98
	 DMAP hexane-CH_2Cl_2	 48b	92
	 NaH THF	 48c	95
	 NaH CH_2Cl_2	 48d	98
	 NaH THF	 48e	85
	 NaH THF	 48f	82

As it is mentioned above the aldehyde e is synthesized since it is an easily hydrolyzable group and can be used for further modifications. For the same purpose, trifluoroacetic acid ester substituted and ethyl ester substituted aldehydes were also synthesized in the similar conditions but they were not used in further steps because their conversion to benzoin was not clean enough compared with the other reactions.

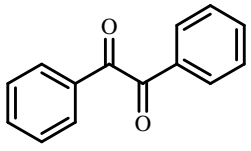
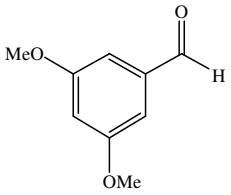
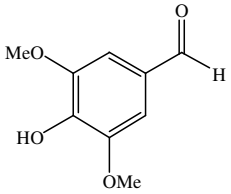
The cyanide ion catalyzed cleavage of benzil is then used for the generation of acyl intermediate and the reaction of this intermediate with the aldehydes synthesized before, furnished the corresponding esters of benzoyl benzoins in good yields. (Scheme 26)

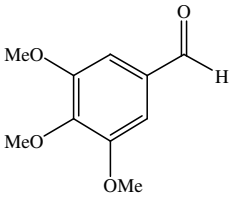
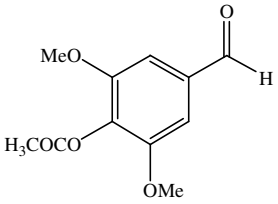
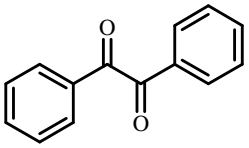
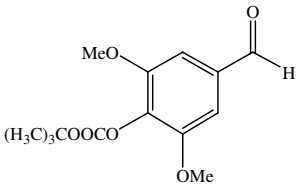
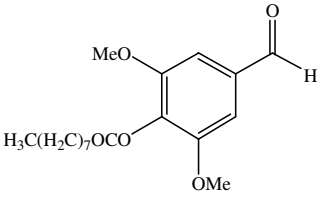
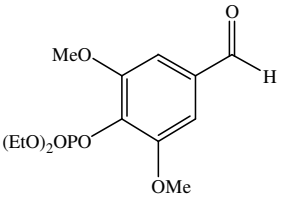


40 47-50 isomer A 51a-g isomer B 52

Scheme 26

Table 2

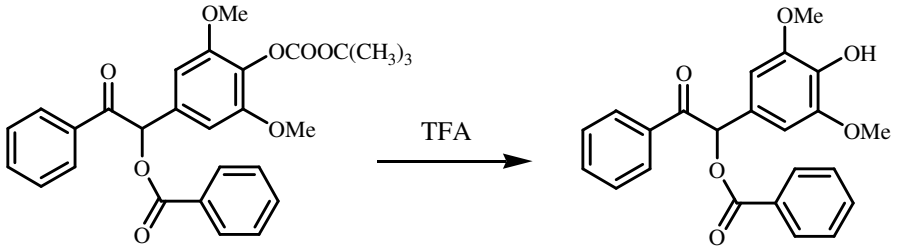
	aldehyde	isomerization ratio A:B	Overall %yield of desired isomer
 40		80:20	51a: 75
		No reaction	51b

		70:30	51c: 60
		85:15	51d: 75
 40		75:25	51e: 70
		60:40	51f: 50
		60:40	51g: 50

The most important drawback of this condensation reaction is that the selectivity of the reaction is not high enough in all cases; the isomer with phenyl ring accomodating substituents adjacent to carbonyl group rather than the hydroxyl group are formed besides the desired isomer (Table 2). The amount of isomerisation can be assigned by crude NMR spectra where the shift values of the two ortho protons of benzoyl moiety resonates at different ppms for each isomer. In such a isomerisation case a further purification with column chromatography is required. The isomers were separated by column chromatography and the NMR spectroscopic data for the products were given in figure 32-38.

The reaction of 3,5-dimethoxy-4-hydroxy benzaldehyde (47) with benzil (40) somehow did not work well. In order to achieve this problem an alternative synthesis was planned. The benzoyl benzoin which have an esily hydrolyzable substituent in the 4th position such as t-butoxy or acetate substituted benzoins were hydrolysed in different conditions in order to obtain 4-OH substituted derivative. The hydrolysis of acetate derivatives (51d, 51g) with DBU in benzene:CH_2Cl_2:MeOH solvent was not a clean reaction, the desired product could not be isolated. Treatment of the acetate derivatives with K_2CO_3 in methanol-water and in another case with piperidine in DMF also lead to unacceptable amount of isomerization.

The hydrolysis of t-butoxide ester (51e) with trifluoroacetic acid in anhydrous CH_2Cl_2 was afforded the desired product (51b) in acceptable yields. The reaction is sensitive to the amount of TFA used by means of isomerization. High amount of TFA may cause benzoin to prefer enol form and so cause isomerisation. After several trials it was found that the cleanest reaction is observed when 1:5 equivalents of TFA is used.

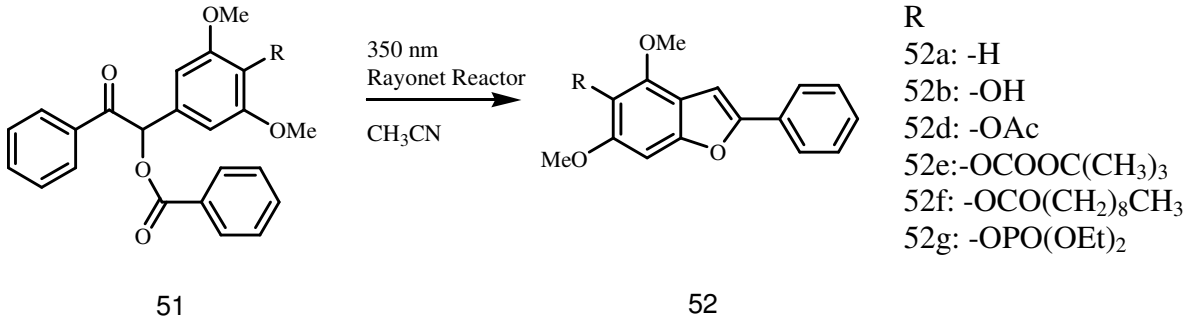


51e 51b

Scheme 27

The new substituents were important for us if and only if they can show photoreleasing ability comparable with the reference molecule 3',5'-dimethoxy benzoyl benzoin when exposed to UV light under the same conditions. The conversion of the 3',5'-dimethoxy acetyl benzoin into the benzofurane analog under 350 nm light was mentioned by Scheenan et al [92] and in the previous parts of this document. The 4-substituted benzoyl benzoins were exposed to light in the Rayonet Reactor and it was

found out that all of them can be converted to corresponding benzofurane derivatives releasing benzoic acid, in almost quantitative yields.



51 52

Scheme 28

A kinetic study was then performed inorder to compare the photoreleasing abilities of the new benzoyl benzoins. The solutions of new derivatives with almost same concentrations were prepared in deuterioted acetonitrile. The solutions were then placed in NMR tubes and exposed to UV light at the same time in our hand made Rayonet reactor. The reaction is then monitored with NMR spectrum acquired for every 15 minutes.

The NMR spectrums of the starting material and the product for the case of 3',5'-dimethoxybenzoin was shown in the figure below. Two characteristics peaks were determined in the spectra, the singlet peak at 6.35 ppm which belongs to the CH of benzoin and the peak at 7.79 ppm which is belongs to the CH's of phenyl group of benzofuran, and the change in the intensity of the those peaks was recorded (Figure 19).

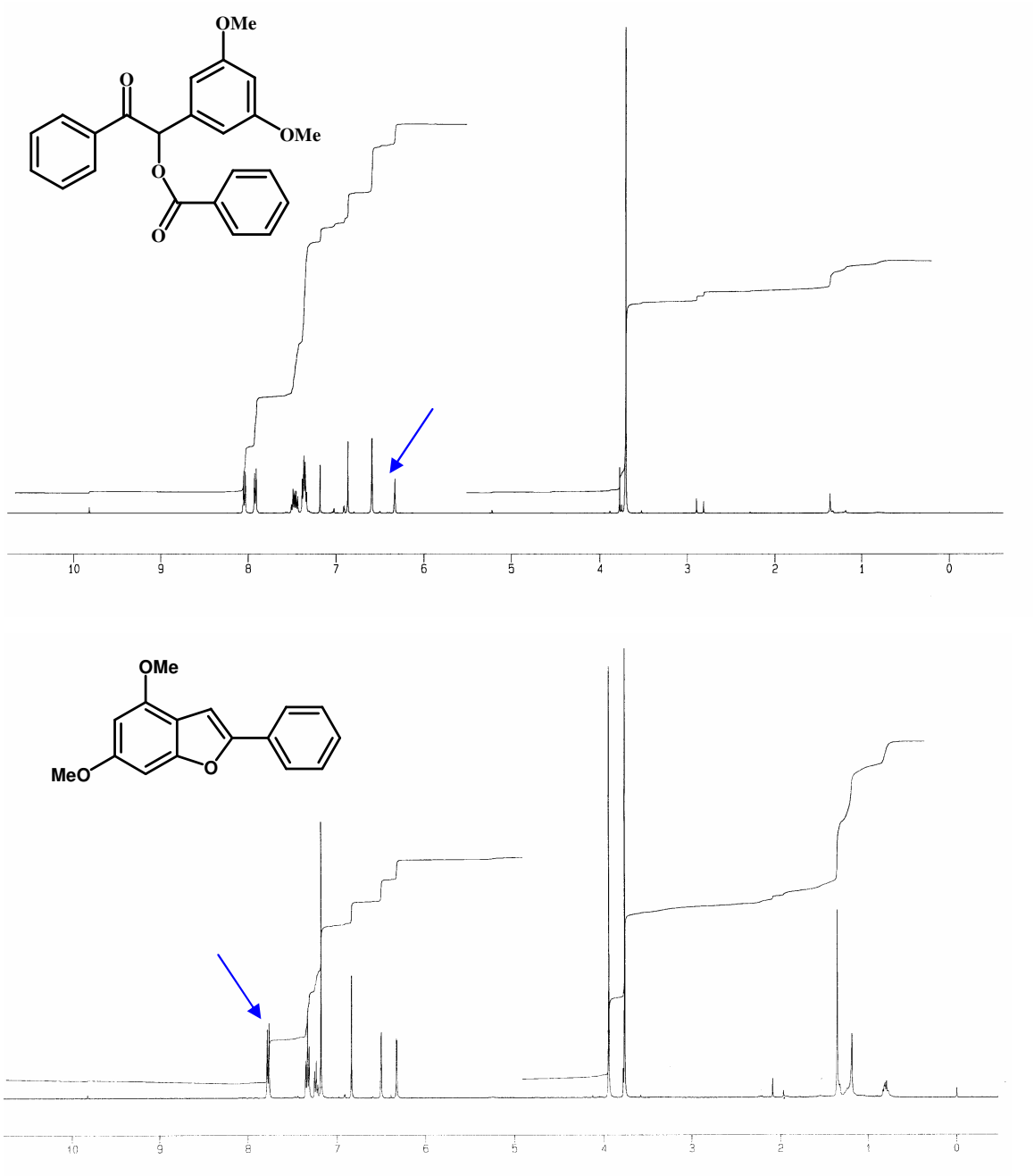


Figure 19: NMR spectra for kinetic study

The apperaence of the dublet at 7.79 ppm can be easily diffrentiated in the crude NMR spectrum as it can be seen in the following figure (Figure 20).

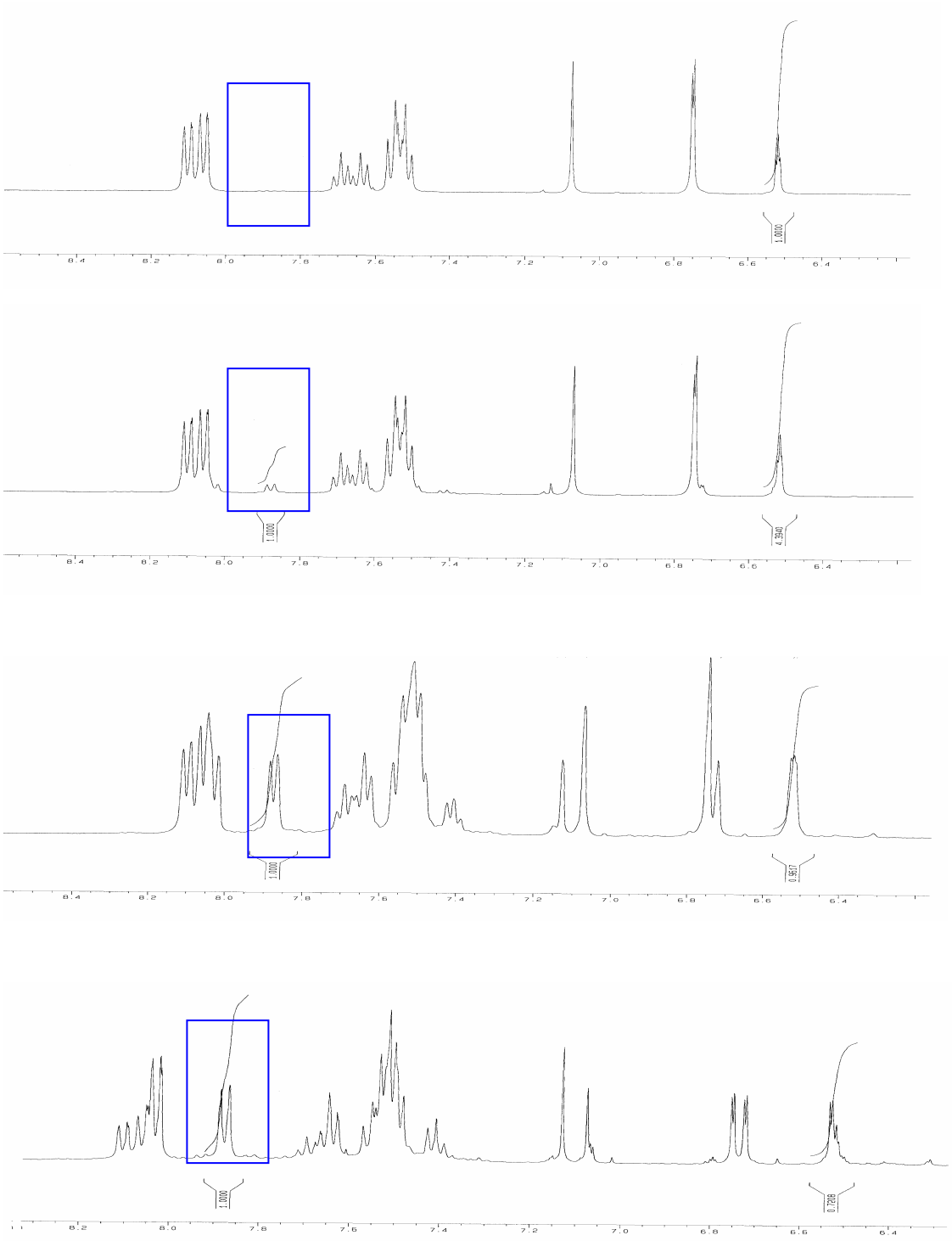


Figure 20: The appereance of the benzofuran peaks in the crude NMR spectrum

The study on the NMR spectra of the reference molecule was applied to the other selected benzoyl benzoins and the kinetics of their photorelease ability was brought to light. The change in the intensity of the peaks was emphasized as logarithmic functions that are exhibited in the graph below (Figure 21).

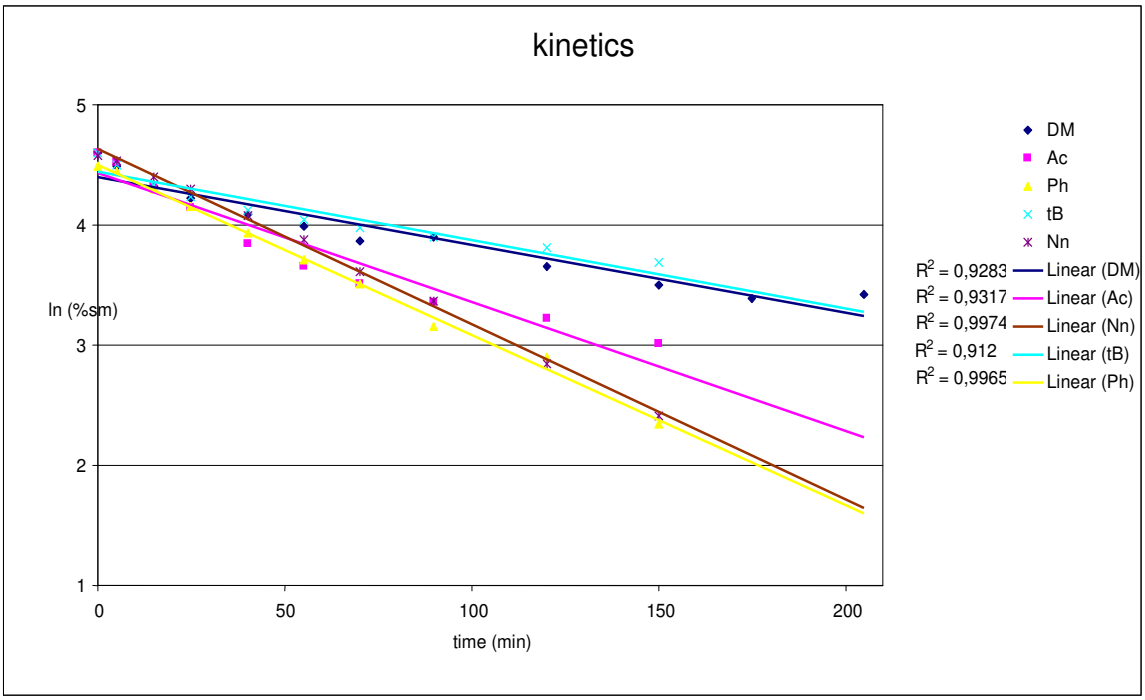


Figure 21: The kinetics photoreleasing abilities of benzoyl benzoins

As it can be seen from the graph all of the derivatives have better or at least same photoreleasing ability compared with the reference molecule. The conversion of the benzoyl benzoins to corresponding benzofuran derivatives were completed almost quantitatively. The products were all purified by column chromatography and assigned by NMR spectroscopy.

The hydrolysis of benzoyl protected benzoins afford the corresponding unsymmetrical 4-substituted benzoins or benzils upon oxidation. Hydrolysis of the products to the corresponding benzoins was carried out in a basic medium. Most of the reactions afforded an isomeric mixture in some cases it was a great problem to achieve.

If the oxygen was not removed from the medium before the reaction, oxidation problem occurred during hydrolysis. According to Demir and Reis[85], isomerization possibly occurs via an endiol and the yellow color developed during the hydrolysis was attributed to this intermediate (Figure 22). This intermediate is expected to be easily oxidized during hydrolysis if air is not excluded from the medium.

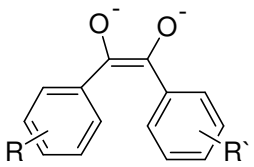
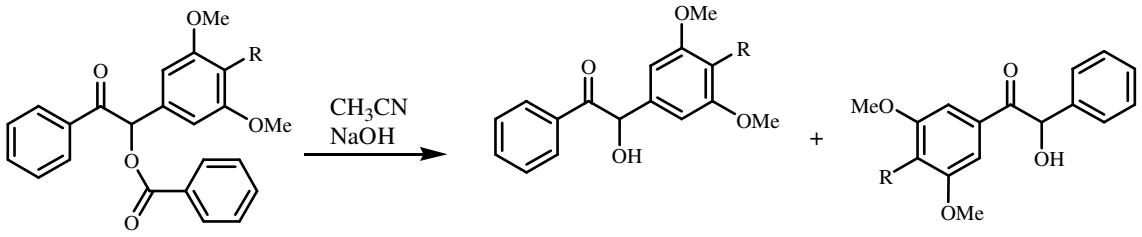


Figure 22: The endiol intermadiate

The reaction conditions and the ratio isomerization of the benzoins were given in the table below. The desired isomers of the benzoins were obtained after the separation of the isomers by column chromatography and the structures of the products were assigned by NMR spectroscopy.



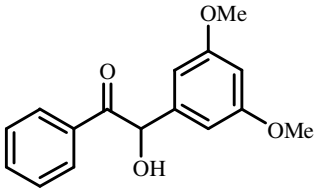
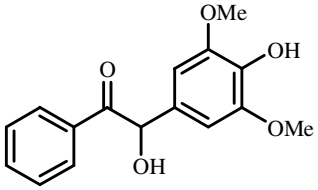
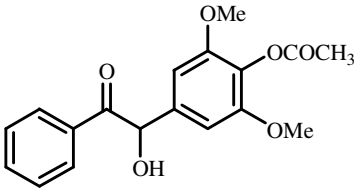
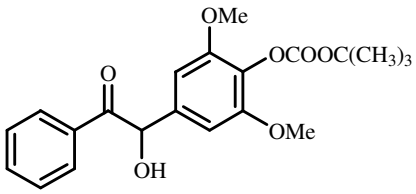
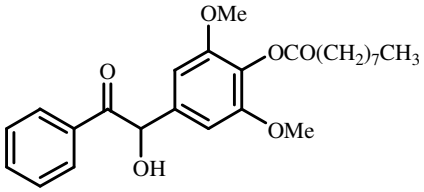
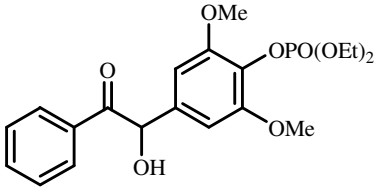
51

isomer A
53

isomer B
54

Scheme 29

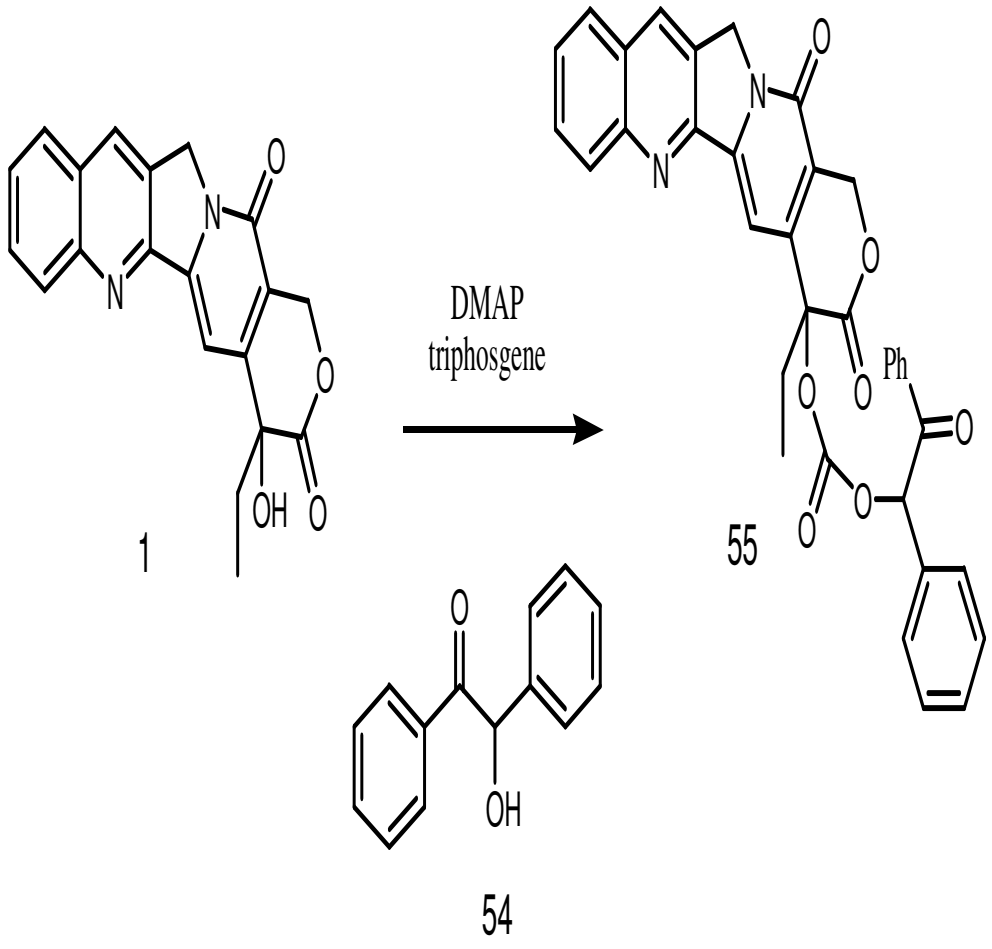
Table 3

product	isomerization ratio A:B	Overall %yield of desired isomer
	70:30	53a: 67
	60:40	53b: 45
	60:40	53c: 60
	55:45	53d: 50
	70:30	53e: 65
	40:60	53f: 37

As it is mentioned above the E-lactone ring of camptothecin including the 20-hydroxyl group is considered to be essential moiety for the drug's activity. Stabilization of this ring via protecting the hydroxy group was studied by many different groups and

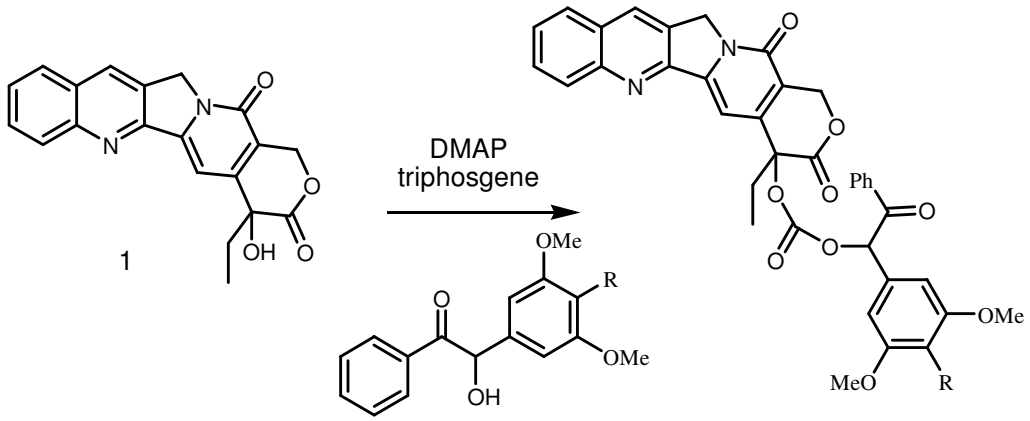
more stable camptothecin analogs were synthesized. In our study the linkage of unsymmetrical benzoins substituted with selected groups addressing the targeting purposes, to camptothecin via a carbonate linker was studied.

For the optimization of reaction conditions reaction of free benzoin with CPT was studied initially. Camptothecin reacted smoothly with triphosgene in the presence of DMAP in dichloromethane to generate, in situ, the highly reactive intermediate chloroformate as it was reported by Zhao et al.[97] The chloroformate –whose formation can be determined by the appreance of pink color- was then reacted readily with free benzoin to give the corresponding CPT-benzoin derivative. (Scheme 24)



Scheme 30

The non-problematic sample reaction did encourage us to move on with the other benzoin derivatives that we have synthesized. The reaction worked well in all cases and the products were obtained in moderate yields. They were purified with column chromatography and their structures were assigned with NMR spectroscopy (Scheme 25).



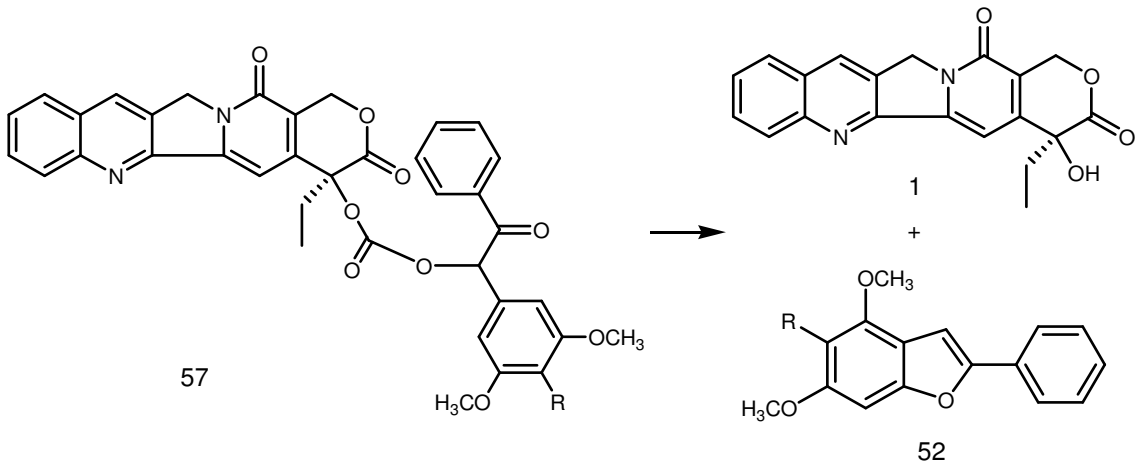
R
57a: -H
57b: -OAc
57c: -OCOOC$(CH_3)_3$
57d -OCO$(CH_2)_8CH_3$
57e: -OPO$(OEt)_2$

Scheme 31

The assignment of ^{1}H-NMR spectroscopy enabled us to characterize the benzoin camptothecin derivatives. The 20-hydroxy peak of a unsubstituted camptothecin appears at 6,5 ppm as a broad singlet. The disapperance of this peak is the most important evidence of the formation of the product. The low field of the spectrum is crowded since the aromatic protons of both benzoin and camptothecin appears at a range between 6,5-8,5. Camptothecin has a characteristic peak at 8.7 ppm which belongs to the proton of 7th carbon. When the integration value of this C-7 peak is compared with the singlet peaks of the protons of dimethoxy substituents (singlet at approximetly 3.7 ppm) in the crude

NMR, the amount of the product and the unreacted camptothecin can be determined. The amount of unreacted camptothecin was in the range of 20-30% of the product and was successfully removed from the product by column chromatography.

The next step of the study was to provide the photoreleasing abilities of the products. The derivatives were subjected to UV light in the Rayonet reactor and the release of CPT by the conversion of the benzoin to benzofurane was observed with TLC.



Scheme 32

As it was mentioned several times before, the selective targeting of a compound to tumors, or for that matter to any structure in the body, requires high stability of the compound in blood. Stability was already the goal of the study so, as the last step of this study, the stability of the new compounds was investigated.

The most currently used analytical method for camptothecin derivatives is high performance liquid chromatography coupled with fluorescence detector.[100] The fluorometric detection method show high sensity due the high fluorescence feature of the totally planar pentacylic structure of camptothecin. The hydrolysis of the lactone moiety in camptothecin is also routinely monitored by using HPLC with fluorescence

dedector. Understanding the pharmacokinetic and pharmacodynamic parameters of lactone ring opening requires methodologies that allow quantitation of the intact lactone species, total drug concentrations and preferably, carboxylate levels as well. HPLC using a C_{18} column is routinely used to achieve separation and quantitation. Several methods have been described for the camptothecins involving quantitation of only the lactone form or for the simultaneous determination of the lactone and carboxylate forms. Method development for HPLC analyses of camptothecins can be very difficult, however, due to the numerous variables that must be considered. In the simplest case of only the lactone separation, two or three components are normally present in the mobile phase. A buffer is used to control the pH of the mobile phase, while acetonitrile is used to control retention of the lactone form of the analogue. A third component is also frequently added to control peak tailing due to interactions between the analyte and underivatized silanols on the column wall. At pH values above 3.5, silanols act as weak acids that have a strong interaction with basic or amino-containing compounds. This interaction results in a dual retention process, which produces peak tailing. Pronounced peak tailing makes quantitation more difficult and less accurate. The addition of a positively charged substance to the mobile phase counteracts this problem by interacting with the underivatized silanols and essentially "coating" the column. In the past, HPLC methods for camptothecin separations have included substances such as sodium dodecyl sulfate (SDS) in the mobile phase to improve peak shape. For the more accurate and less laborious simultaneous determination of the lactone and carboxylate forms of a camptothecin, an additional component, namely an ion-pairing agent, must also be employed. Without this ion-pairing agent, the chromatographic behavior of the charged carboxylate species is such that it will not be retained on the C_{18} column to an appreciable extent (i.e. the carboxylate elutes in the void volume), precluding simultaneous separation of the lactone and carboxylate form of the drug. The ion-pairing agent tends to mask the charge of the camptothecin carboxylate species, increasing its affinity for the column.[101] Common ion-pairing reagents such as tetrabutylammonium phosphate are normally added to a buffered mobile phase to facilitate the separation. Method development for the analysis of camptothecins using HPLC can therefore be very time-consuming and complicated. Normally the following three or four parameters

must be optimized for a successful separation: Type and concentration of ion-pairing reagent; type, concentration and pH of buffer; amount of organic modifier and concentration of SDS or other agent capable of masking silanols. In their study Burke et al, documented a HPLC methodology for the simultaneous separation of the lactone and carboxylate forms of numerous camptothecin analogues, employing only a simple two component mobile phase system consisting of triethylamine acetate (TEAA) buffer and acetonitrile. In their application, triethylamine acts as the ion-pairing agent, masks underivatized silanols and is the major buffer component. By altering only the composition of the TEAA buffer with respect to acetonitrile (rather than three or four separate components), method development time was markedly reduced. In our study, we have used their methodology for the simultaneous separation of the lactone and carboxylate forms of the camptothecin derivatives (figure 23).

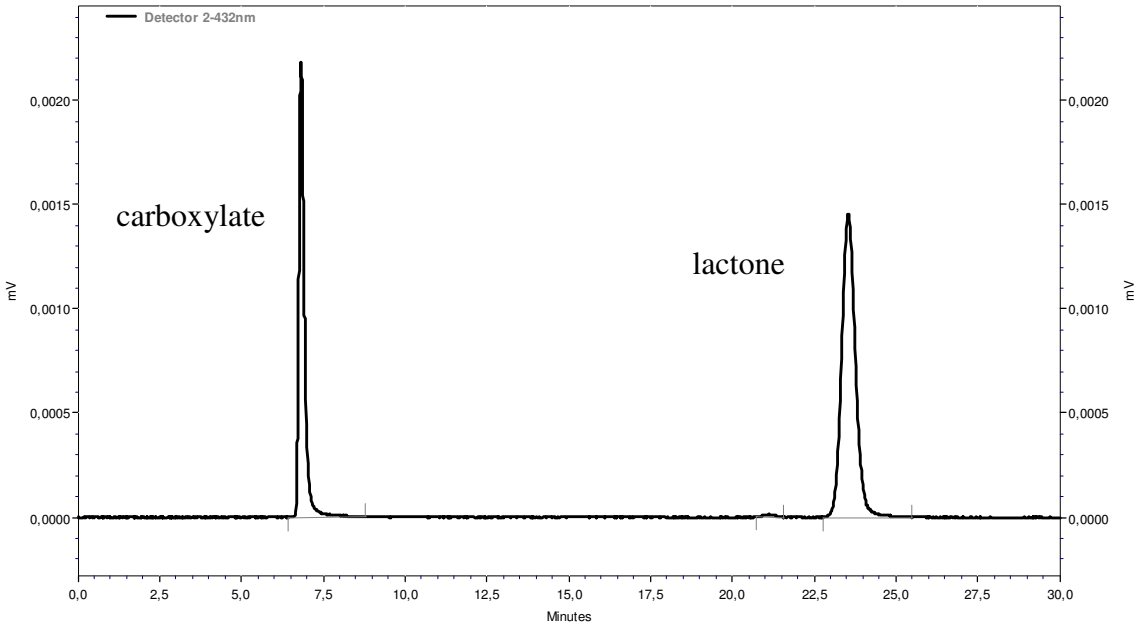


Figure 23: HPLC separation of camptothecin carboxylate and lactone species was performed by the mobile phase consists of 23:77 (v/v) acetonitrile-triethylamine acetate buffer (1%, v/v), pH 5.5.

The applied method altered the retention of lactone species of camptothecin and the early elution of carboxylate species on the reverse phased C_{18} column. The area under the peaks was measured and their diffrentiation was calculated. (Figure 24)

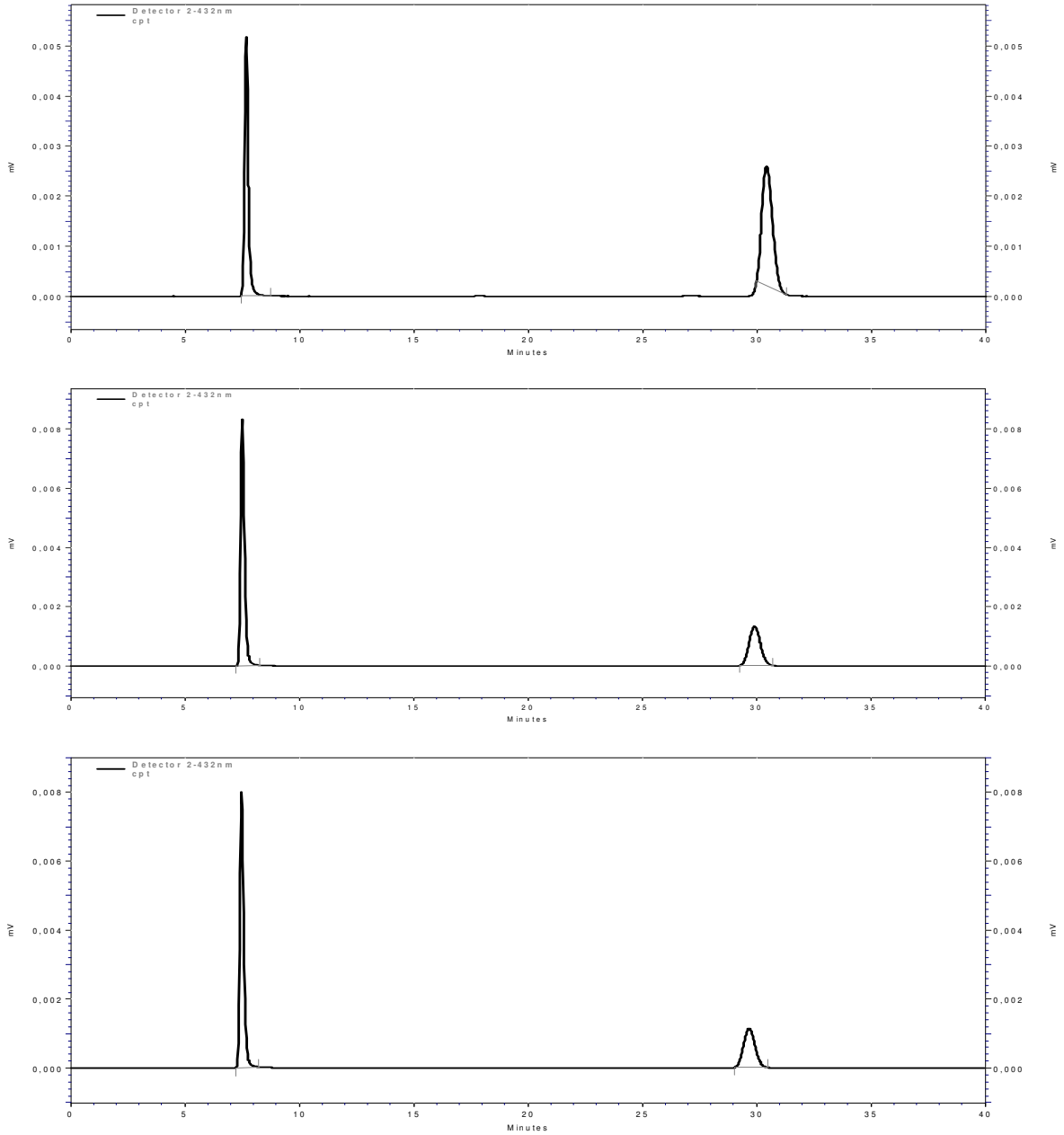


Figure 24: The series of HPLC spectra recorded

The data obtained by HPLC monitoring of the stability of the drugs in PBS buffer pH=7.4 and their best fitting curves are shown in the figure 25. It is clear that, modification of CPT at the 20th position with benzoin derivatives enhances the drug stability: by 3 hours CPT lactone level fell less than 50% whereas CPT benzoin lactone level reached only 70% at the same time.

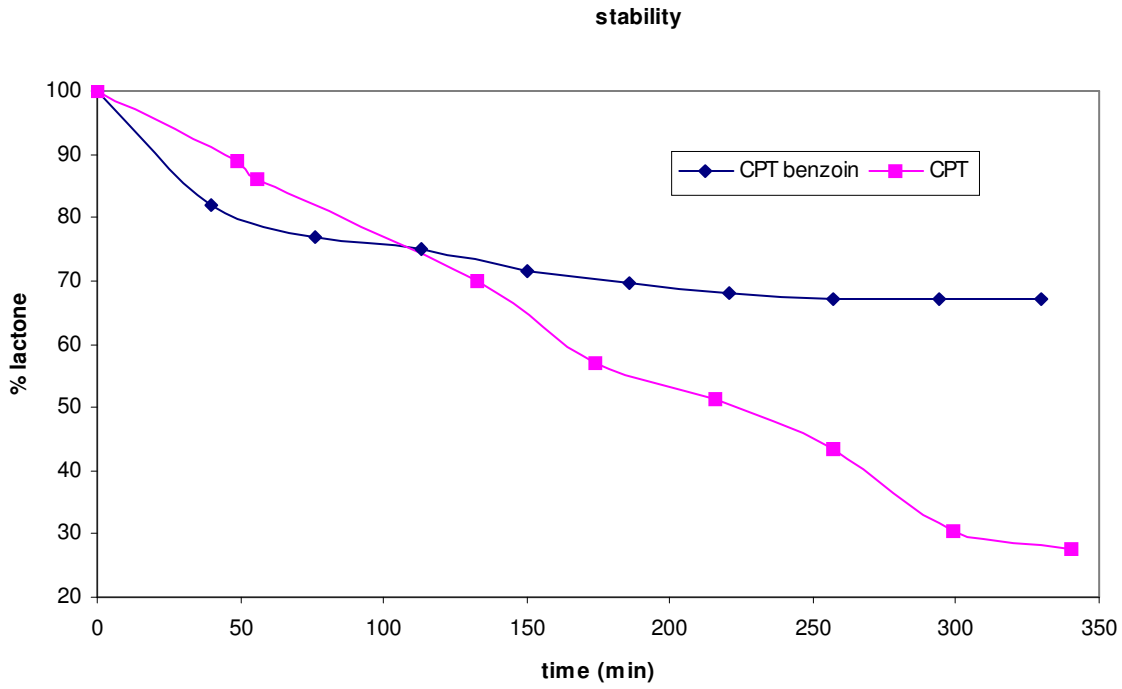


Figure 25: Stability of CPT prodrugs

As a result, this part of the camptothecin study was completed succesfully addressing the three major aim of the designed molecule:

- The substituents were successfully connected to camptothecin through the 20-hydroxyl functionality which provides the protection of hydroxyl group. Thus, camptothecin derivatives that are more stable than camptothecin itself was synthesized.
- The substituents have also improved the solubility of camptothecin which was the most impotant handicap in its applications.

- The substituents on the benzoin part of the molecule are suitable and representative molecules that can be used in targeting purposes. For example the phosphate derivative showed that a DNA segment can be connected to camptothecin in further studies. Succesful connection of the acetyl group can be imitated in the future work for the connection of sugar groups on camptothecin.

2. 2 Modifications on the C-7 position of CPT

As it was mentioned in the introduction part, by means of modification, the most suitable part of the molecule is the A-B quinoline ring where wide possibilities of variation exists especially in positions 7, 9, 10 and 11. To enhance stability of the ternary cleavable complex and determine the binding site, CPT derivatives with an alkylating group have been synthesized and was tested against mammalian topo I. [102] As a result of the study, it was found that most substitutions at the 7th position of the B ring of CPT enhance topo I inhibition. When 7th substituted CPT's were examined according to the model below, they all showed more favorable interactions than did CPT itself.

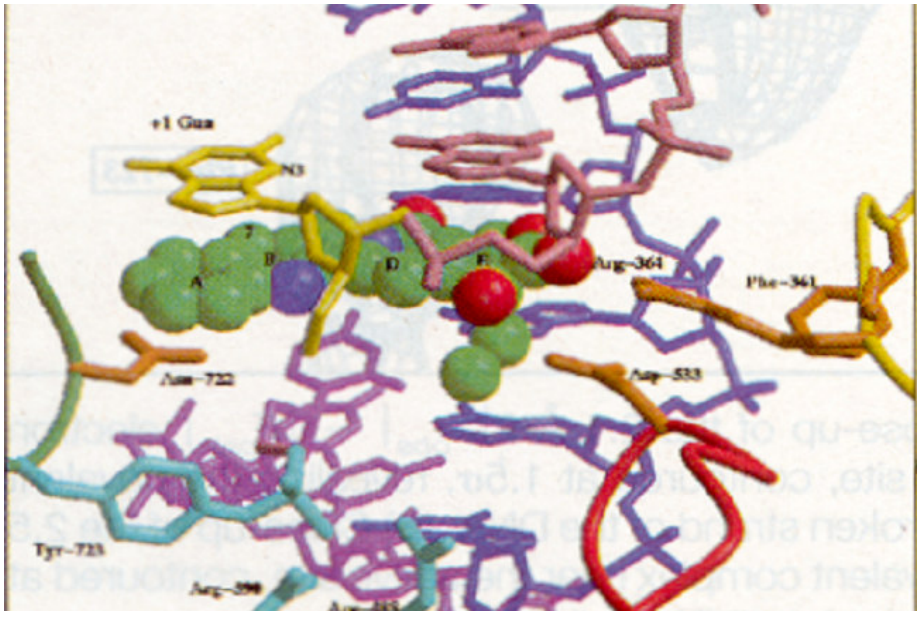


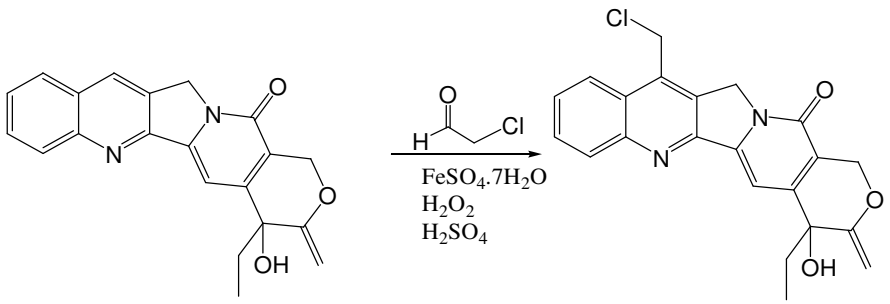
Figure 26: Interactions between CPT-DNA-TOPO I [108]

Fan et. al.[102] suggest that electron rich groups of moderate size would be favored for both the 10th and 11th positions whereas 7th and 9th positions could bear a bulky group.

To date, the only CPT analogues approved for clinical use, that is, topotecan (3) and irinotecan (4), are derivatives with substitutions within the quinoline ring. In our early studies, substitutions on the C-7 position of camptothecin were concerned. The modification was designed to provide high anti-tumor activity, water solubility and low toxicity.

Recent studies have indicated the importance of lipophilic groups in position 7 for potent CPT activity.[103] The scarce solubility in water of these compounds does not represent a disadvantage, due to the possibility of successful administration of CPT derivatives. The 7-amine group substituted CPT and 7-silyl CPT derivatives found to show potencies of antitumor activity comparable to or better than CPT itself. [104]

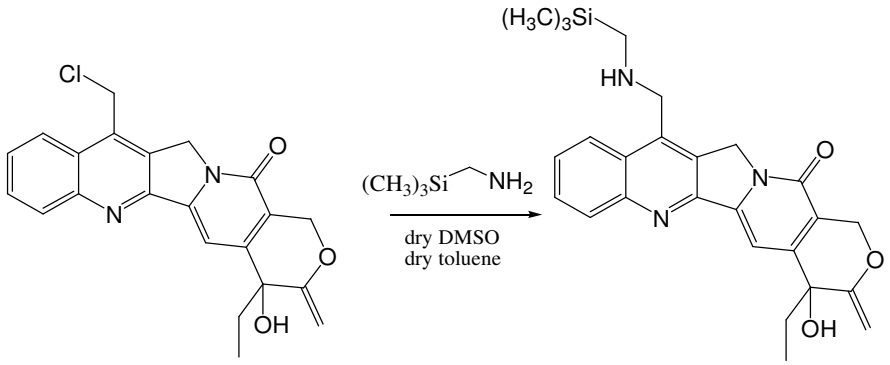
The initial studies involves the substitution of C-7 position with silyl-amino functions to improve the stability , solubility and anti-cancer activity of the molecule. For this purpose first the C-7 haloalkyl substituted CPT derivative was synthesized as a starting material for the synthesis of silyl amine derivatives. As pointed out in the literature[105], homolytic alkylation of heteroaromatic bases can be performed in aqueous acidic media: alkyl radicals attack to the electron-deficient positions of the protonated bases. This reaction was found to be convenient for modification of CPT to overcome its insolubility in organic solvents. Furthermore the reaction was expected to occurred selectively at position 7. Treatment of CPT with hydrogen peroxide and ferrous sulfate in aqueous sulfuric acid furnished 7-chloro CPT. (scheme 33)



1 58

Scheme 33

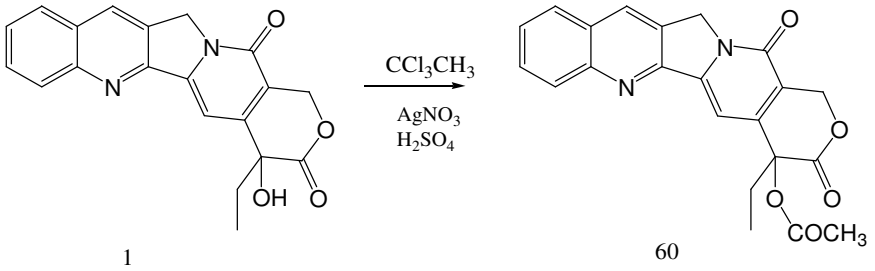
The obtained C-7 chloromethyl CPT was then reacted with trimethylsilyl methyl amine, to obtain a new silyl amine derivative of CPT. (Scheme 34) It was a difficult process to purify the product but it was succedded after several column chromatographies.



1 59

Scheme 34

The difficulty in the purification step reflected negatively to the overall yield of the synthesis. To overcome the problem or at least to minimize the loss of product in the column chromatography, 20-hydroxy group of camptothecin was acetylated according to the procedure reported by Cao et al. before the chloroalkylation step.[106] (Scheme 35) The reaction was performed with trichloro ethane and silver nitrate and the product was obtained in moderate yield.

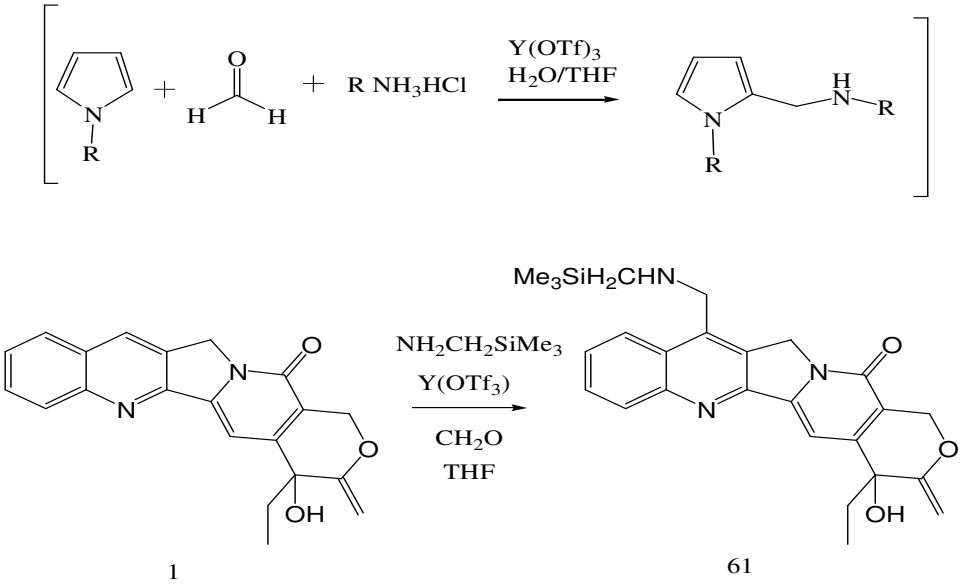


1 60

Scheme 35

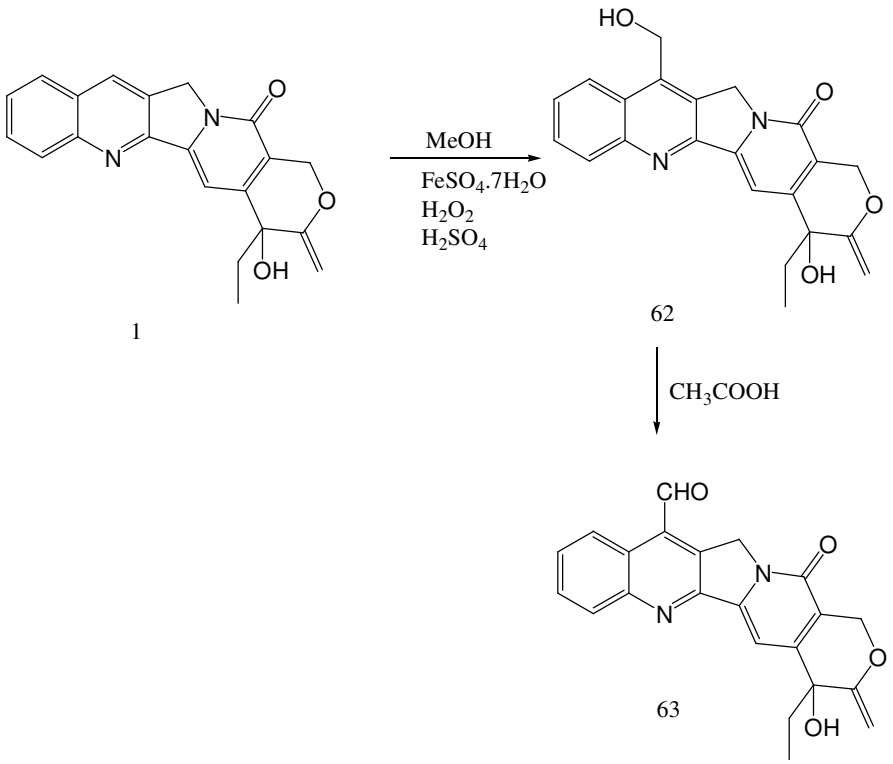
20-acetyl CPT (60) is then reacted with chloroacetaldehyde in the same conditions to obtain 7-chloro CPT (58), but the separation of the product was not easier than the non-acetylated 7-Cl CPT. That is why, the procedure was not applied in further studies.

As an another alternative for the introduction of a silyl substituted secondary amine in position 7, the Mannich reaction route was followed. For this purpose first N,N-trimethylsilyl methylamine was prepared with known simple procedures. However, no product was obtained by the classical Mannich reaction performed by using formaldehyde. A study of Zhang et al. suggests that yttrium triflate is an effective catalyst for the Mannich reaction between primary amine salts, aldehydes and N-alkoxycarbonylpyyrole.[107] They obtained the product in mild conditions with moderate yields. Inspiring from that reaction, amine alkylation of CPT was tried by using the same reaction conditions. However, the reaction of CPT with silyl amine compounds, catalyzed by yittrium triflate gave no trace of the product. (Scheme 36)



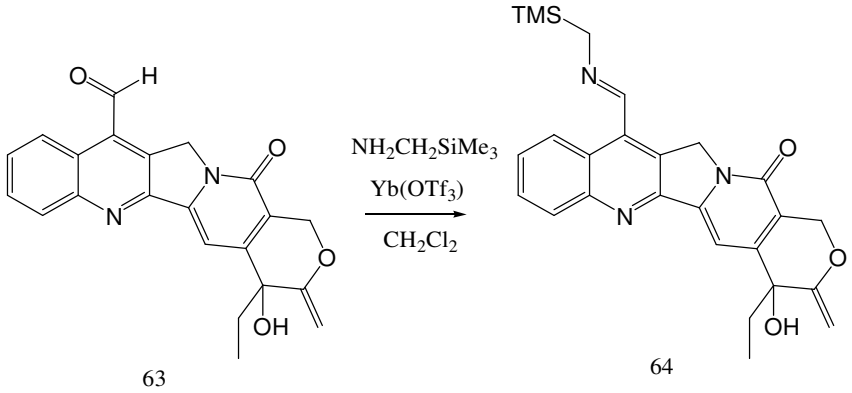
Scheme 36

Sawada et al has reported another way for the modification of C-7 position; conversion of of CPT to 7-formyl CPT and then to 7-amino derivatives.[108] According to their procedure CPT was first converted to 7-hydroxy methyl CPT (62) in aqueous acidic media and the addition of $FeSO_4.7H_2O$ and hydrogen peroxide. Addition of water to the reaction mixture after 14 hours results in the precipitation of product which was filtered through a celite pad. 7-CH_2OH CPT (62) was then refluxed in acetic acid and oxidized to 7-formyl CPT (63) which was purified by flash column chromatography. (Scheme 37)



Scheme 37

Conversion of 7-formyl CPT (63) to the 7-imino derivatives was performed by using $Yb(OTf)_3$ catalyzed condensation reported by Dallavalle et al.[109] The product which was purified by column chromatography was obtained in moderate yield (Scheme 38).



Scheme 38

As another branch of the modification studies, connection of CPT with gold nanoparticles was concerned. In literature there are many examples of oligonucleotide-gold nanoparticle conjugates serve as recognizing specific sequences in DNA segments.[110] Biologically active compounds can also be binded to gold nanoparticles reversibly and then, can be used in controlled release of drug systems. Gold nanoparticles can be bound to molecule if there is a sulfur functional group on the molecule. Thus, initially functionalizing of CPT with sulfur group was studied.

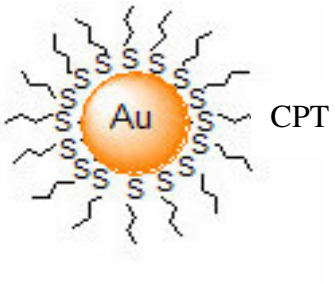
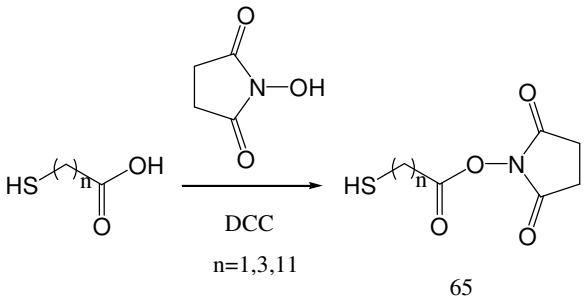


Figure 27: CPT connected to a gold nanoparticle

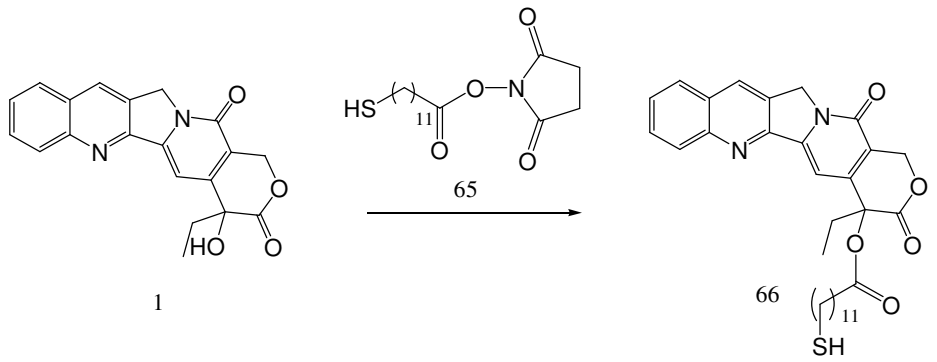
Esterification of the hindered tertiary alcohol on the 20th carbon of CPT can not be achieved by classical esterification processes. In literature there is an example in which CPT was converted to its ester derivative with the t-Boc protected N-hydroxy succinimide (NHS) esters of chiral amino acids by using $Sc(OTf)_3$ and DMAP.[111] Thus, in order to be able to apply this procedure for our synthetic purposes, derivatives of mercapto carboxylic acids were first converted to their NHS esters. (Scheme 39)



65

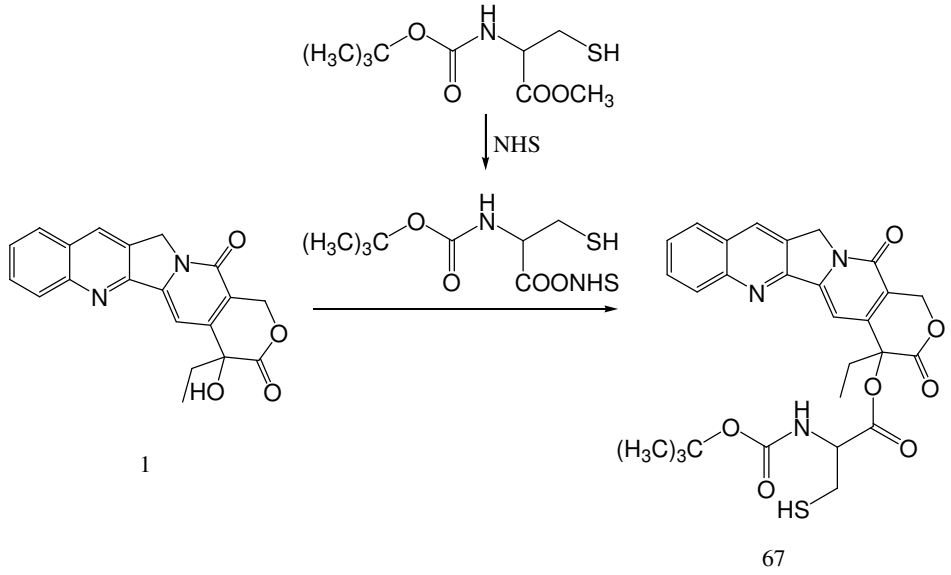
Scheme 39

The best chemical yield was obtained from the synthesis of 11-mercapto undecanoic ester. It is then reacted with CPT by using $Sc(OTf)_3$ and DMAP but the product could not be purified. (Scheme 40)



Scheme 40

t-Boc cysteine methyl ester was also converted to its NHS ester and reacted with CPT in the same conditions but this reaction was also failed. (Scheme 41)

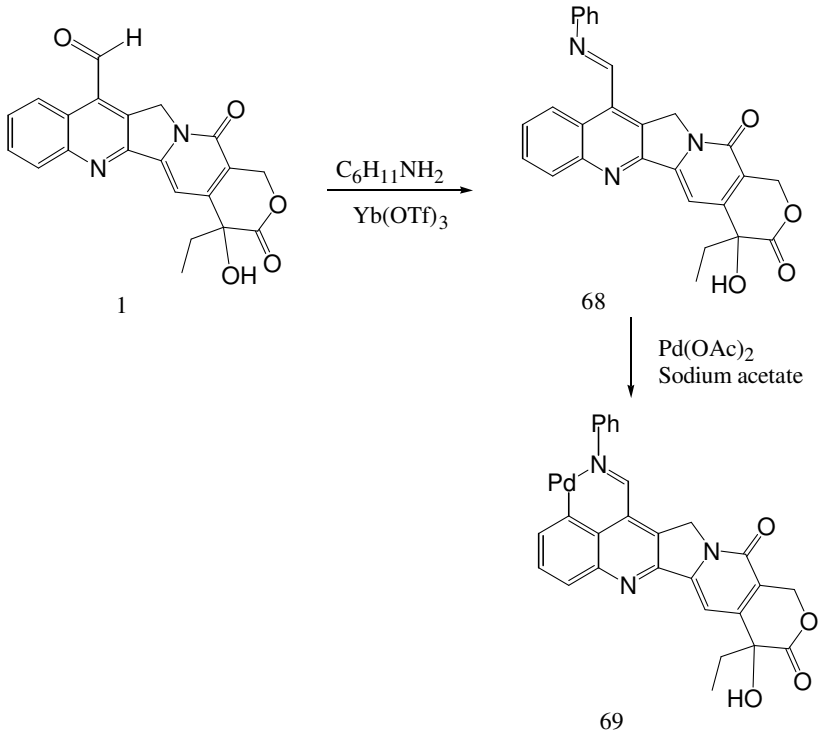


Scheme 41

In literature there are many articles concerning five or six membered cyclometallated complexes having nitrogen, phosphorus, oxygen or sulfur donor atoms.[112] This idea inspired the study since an imine derivative of CPT can be converted

to six membered cyclometallated complex. The first trial was done with palladium metal.

First the 7-formyl CPT was converted to an imine derivative by reacting with cyclohexylamine. Then the imine was reacted with palladium acetate to obtain the desired product. (Scheme 42)



Scheme 42

The desired product unfortunately could not be isolated but if this reaction could be achived any ligand would be introduced on to palladium for further functionalization. Besides, if the complex could be formed with platinium metal, a cisplatin-CPT complex could also be formed. The reaction can be studied in the future since it is a promising type of reaction.

Another branch of the study involves the idea of connecting CPT and cisplatin. Cisplatin [*cis*-dichlorodiammine platinium (II)] is one of the most effective anticancer

agents currently available for the treatment of the testicular, ovarian and bladder cancers.[113] In addition, cisplatin is widely used in combination with other anticancer agents in treating several cancer types. However, the adverse effects that are observed in patients receiving cisplatin and neurotoxicity as well as the low activity for certain kinds of cancers, strongly limits its clinical use. Furthermore, the development of acquired resistance to cisplatin is frequently observed during chemotherapy. In order to overcome these drawbacks of cisplatin, numerous analogues have been synthesized and evaluated to develop alternative active agent with equivalent or greater antitumor activity and lower toxicity than cisplatin. One of the most effective derivative of cisplatin is carboplatin which is also currently used to treat testicular and ovarian cancer.

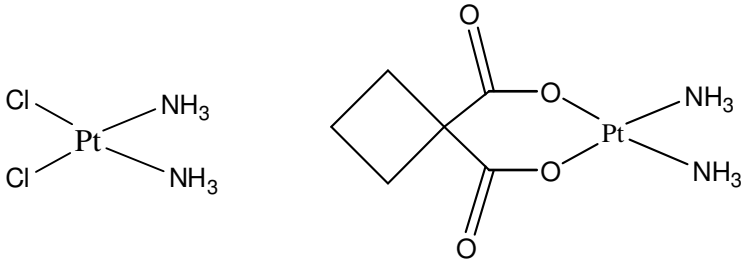
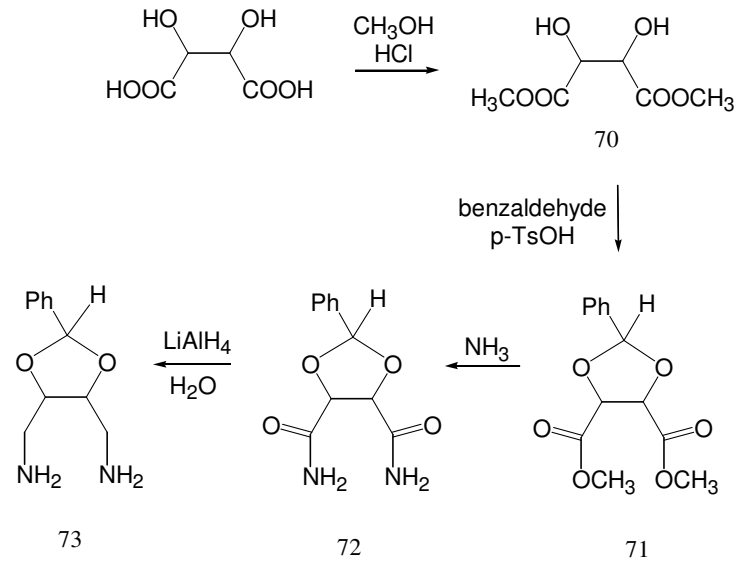


Figure 28: Cisplatin and carboplatin

Connection of cisplatin with CPT may overcome the existing application problems of both drugs and may exhibit a dual effect on the treatment of some cancer types.

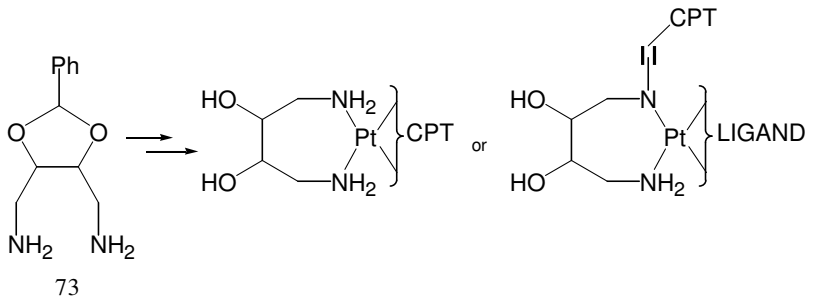
In efforts to develop new platinium complex, a diamine compound was synthesized initially starting from tartaric acid. (Scheme 43)



Scheme 43

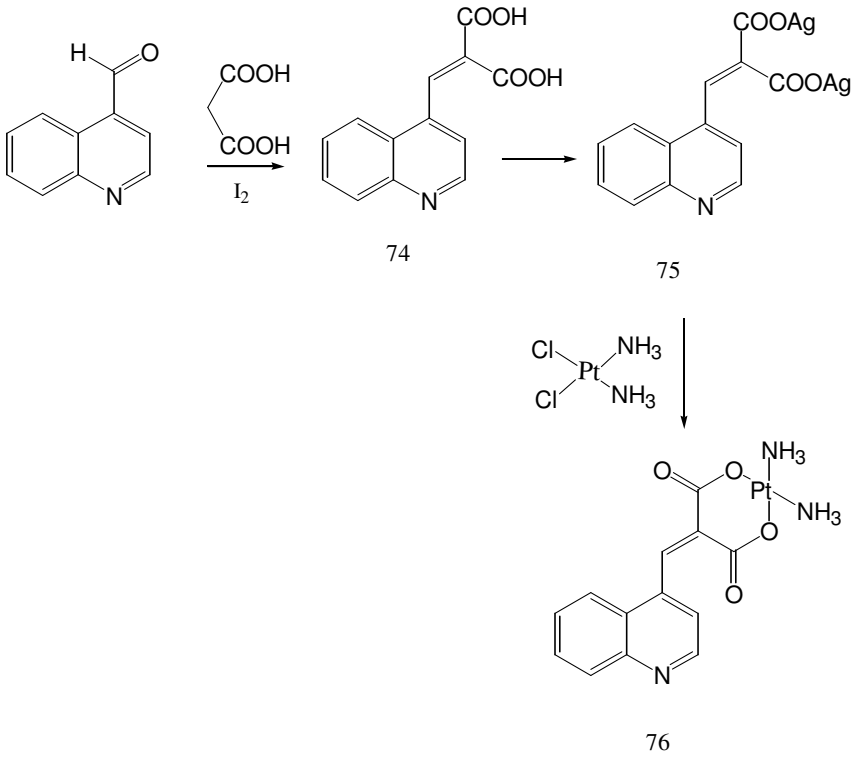
Tartaric acid was first converted to its methyl ester by treating with methanol, followed by the protection of diol by benzaldehyde. The ester is then saturated with ammonia gas in order to obtain amide product. Lastly the reduction of amide by lithium aluminium hydrate gave the desired diamine with the protecting group. In each step of the reaction the chemical yield was higher than %90 except the reduction step.

Conversion of the diamino compound to cisplatin and then the connection of CPT to this compound as it is shown in the scheme below could not be achieved just because it can not be tried several times. But these kinds of reactions are also very promising and it is going to be completed in the future.



Scheme 44

As a last intend to synthesize cisplatin-CPT complex Knoevanagel reaction of 7-formyl CPT with malonic acid was planned. But before CPT, quinoline aldehyde was used in the reactions as a model reaction. Quinoline aldehyde was first converted to disilver salt and then reacted with cisplatin and the formation of the product was assigned by crude NMR. The product could not be isolated. The reaction was described in the scheme 45.



74 75 76

Scheme 45

The reaction was then performed on CPT molecule but the reaction was failed in the last step, there was no formation of CPT-cisplatin complex.

CHAPTER 3

EXPERIMENTAL

NMR spectra were recorded on a Bruker DPX 400. Chemical shifts δ are reported in ppm relative to $CHCl_3$ (^1H: δ=7.26) and $CDCl_3$ (^{13}C: δ=77.0) as an internal standard; coupling constants are reported in Hz. Column chromatography was conducted on silica gel 60 (mesh size 40-63 um). TLC was carried out on aliminium sheets precoated with silica gel $60F_{254}$ (Merck) and the spots were visiualized with UV light (λ=254 nm). HPLC spectra were recorded on Thermo Finnigan HPLC with Fluorescence dedector.

3.1 20-OH Substituted Camptothecin Derivatives

3.1.1 Synthesis of the Aldehydes:

Benzaldehyde derivatives a, b and c were purchased (Aldrich) and used as obtained. The others were prepared from 3,5-dimethoxy-4-hydroxy benzaldehyde as it is described below:

i. 4-Formyl-2.6-dimethoxyphenyl Acetate 48a:

To a stirred solution of 5 mmol 3,5-dimethoxy-4-hydroxy benzaldehyde and 3 equivalent pyridine in 10 ml CH_2Cl_2 were added 2 equivalent acetyl chloride and the solution was stirred in an ice bath. The mixture was then stirred at room temperature for one further hour and completion of the reaction was TLC monitored. The CH_2Cl_2 was evaporated and 1:3 EtOAc:hexane was added to the solution. Then the mixture was filtered through a pad of silica and the product was obtained in pure form. (MW: 224.21)
^{1}H NMR ($CDCl_3$) δ 2.28 (3H,s), 3.84 (6H,s), 7.05 (2H, s), 9.81 (1H,s)

ii. 4-Formyl-2.6-dimethoxphenyl pivalate 48b:

To a solution of 5 mmol 3,5-dimethoxy-4-hydroxy benzaldehyde in 20 ml hexane and 25 ml CH_2Cl_2 were added 12 equivalent dicarbonate. The cloudy solution was cleared by addition of 0.05 equivalent of DMAP. The reaction was TLC monitored and after completion of the reaction the solution was extracted with 1N HCl and water. The product was purified with column chromatography with1:4 EtOAc:Hexane as eluent. MW: 266.29, white solid
^{1}H NMR ($CDCl_3$) δ 1.49 (9H, s), 3.87 (6H, s), 7.06 (2H, s), 9.82 (1H, s)

iii. 4-Formyl-2.6-dimethoxyphenyl nonanoate 48c:

To a solution of 5 mmol 3,5-dimethoxy-4-hydroxy benzaldehyde in 30 ml anhydrous THF 1.2 equivalent NaH and 2 equivalent nonanoyl chloride were added under argon atmosphere. The reaction was TLC monitored and after completion of the reaction the solution was extracted with $NaHCO_3$ solution. The product was purified with column chromatography with 1:3 EtOAc:Hexane eluent. MW: 322.39 yellow oily solid

^{1}H NMR ($CDCl_3$) δ 0.82 (3H, t, J=5.17 Hz), 1.25 (8H, m), 1.37 (2H,m), 1.69 (2H, p, J=7.44 Hz), 2.52 (2H, t, J=7.36 Hz), 3.79 (6H,s), 7.01 (2H,s), 9.76 (1H, s); ^{13}C NMR ($CDCl_3$) δ 14.5, 23.0, 25.4, 29.4, 29.6, 29.7, 32.2, 34.1, 56.5, 96.5, 106.3, 125.6, 129.3, 134.2, 134.6, 153.2, 170.7, 190.8

iv. Diethyl 4-formyl-2,6-dimetoxyphenyl Phosphate 48d:

To a stirred solution of 2 mmol 3,5-dimethoxy-4-hydroxy benzaldehyde in 5 ml anhydrous CH_2Cl_2 were added 1.3 equivalent NaH in 2 ml of CH_2Cl_2. After 15 minutes 1.2 equivalent diethylchlorophosphate in 2 ml of CH_2Cl_2 were added dropwise to the resulting suspension. The reaction was TLC monitored and after completion the solution was washed with $NaHCO_3$ and water. The product was purified with a column chromatography with 3: 2 EtOAc:Hexane as eluent. (MW: 318.26)

^{1}H NMR ($CDCl_3$) δ 1.34 (6H, t, J=7.53 Hz), 3.87 (6H, s), 4.25 (4H, dq, J=0.6, 7.07 Hz), 7.05 (2H,s), 9.79 (1H,s); ^{13}C NMR ($CDCl_3$) δ16.5, 16.6, 56.6, 64.6, 64.7, 96.5, 106.7, 133.5, 133.6, 135.0, 153.0, 153.1, 190.6

v. 4-Formyl-2.6-dimethoxyphenyl 2,2,2-Trifluoroacetate 48f:

To a solution of 5 mmol 3,5-dimethoxy-4-hydroxy benzaldehyde in 20 ml anhydrous THF 1.2 equivalent NaH and 2 equivalent trifluoroaceticanhydride were added under argon atmosphere. The reaction was TLC monitored and after completion of the reaction the solution was extracted with $NaHCO_3$ solution. The obtained product was pure enough, there was no need for further purification. MW: 278.18

ND190white solid;

^{1}H NMR ($CDCl_3$) δ 3.88 (6H, s), 7.09 (2H, s), 9.84 (1H, s)

vi. 4-Formyl-2.6-dimethoxyphenyl Propionate 48g:

To a solution of 5 mmol 3,5-dimethoxy-4-hydroxy benzaldehyde in 20 ml anhydrous THF 1.2 equivalent NaH and 2 equivalent ethylchloroformate were added under argon atmosphere. The reaction was TLC monitored and after completion of the reaction the solution was extracted with $NaHCO_3$ solution. The obtained product was pure enough, there was no need for further purification. MW: 238.24

3.1.2 General Procedure for the Synthesis of Protected Benzoins

Benzil was purchased from Aldrich and used as obtained in the synthesis of benzoyl protected benzoins. To a solution of 5mmol benzil and 5mmol of aromatic aldehyde in 3 ml DMF was added 0.2 equiv of KCN (the course of reaction generally was not affected by the amount and source of DMF). The reaction was monitored by TLC. After completion of reaction, the mixture was directly subjected to chromatography through to a pad of silica to remove DMF and side products and eluted with 1:7 EtOAc:Hexane. Evaporation of the solvent with rotary evaporator followed by high vacuum furnished the desired product. For some reactions, the product was pure enough for most purposes. Otherwise, products were purified with a second column chromatography with 1:7 EtOAc:Hexane as eluent or crystallized from a mixture of EtoAc:Hexane. In the case of phosphanate derivatives 1:7 EtOAc:Hexane system is not suitable for purification, so DMF was removed with high vacuum and then the product was purified by column chromatography with 1:1 EtOAc:Hexane as eluent.

i. 1-(3,5-Dimethoxyphenyl)-2-oxo-2-phenylethyl benzoate 51a:

MW: 376.402, White solid

^{1}H NMR ($CDCl_3$) δ 6.35 (1H,s), 6.62 (2H,s), 6.90 (1H,s), 7.33 (4H, q, J=7.70 Hz), 7.49 (2H, m), 7.92 (2H, d, J=7.62 Hz); ^{13}C NMR ($CDCl_3$) δ 55.8, 78.3, 101.5, 107.1, 128.8, 129.1, 129.2, 129.8 130.4, 133.8, 133.9, 135.1, 136.1, 161.6, 166.3, 193.9; Anal. Calcd. for $C_{23}H_{20}O_5$

ii. 1-(4-Hydroxy-3,5-dimethoxyphenyl)-2-oxo-2-phenylethyl 51b:

To a suspension of 1 equivalent t-Boc benzoyl benzoin in CH_2Cl_2 5 equivalent trifluoroacetic acid was added. The reaction was TLC monitored and after completion of the reaction the product was purified by column chromatography with 1:3 EtOAc:hexane. MW: 532.62 white solid

^{1}H NMR ($CDCl_3$) δ 3.82 (6H, s), 6.26 (1H,s), 6.67 (1H,s), 6.89 (1H, s), 7.35 (4H,s), 7.46 (2H,s), 7.90 (2H, d, J=7.44 Hz), 8.04 (2H, d, J=7.36 Hz)

iii. 1-(3,4,5-Trimethoxyphenyl)-2-oxo-2-phenylethyl benzoate 51c:

MW: 406.428) white solid

^{1}H NMR ($CDCl_3$) δ 3.78 (3H,s), 3.79 (6H, s), 6.66 (2H, s), 6.93(1H, s), 7.37 (4H,m), 7.50 (2H, m),7.92 (2H, d, J=7.31 Hz), 8.05 (2H, d, J=7.32 Hz)

iv. 1-(3,5-Dimethoxy-4-acetoxy)-2-oxo-2-phenylethyl benzoate 51d:

MW: 434.438 white crystal

^{1}H NMR ($CDCl_3$) δ 2.23 (3H, s), 3.75 (6H,s), 6.69 (2H,s), 6.91(1H,s), 7,48 (2H,dd, J=2.18,7.57 Hz) 7.39 (4H, dt, J=1.92, 6.03 Hz), 7.92 (2H, d, J=7.27 Hz), 8.04 (2H, d, J=7.23 Hz); ^{13}C NMR ($CDCl_3$) δ 20.8, 56.5, 78.0, 96.5, 105.8, 128.7, 129.1, 129.2, 129.8, 129.9, 130.9, 132.1,133.6, 133.8, 135.3, 153.0, 166.1, 168.2, 193.6,

v. 1-(3,5-Dimethoxy-4-tert-butoxy)-2-oxo-2-phenylethyl benzoate 51e:

MW: 604.73 white solid

^{1}H NMR ($CDCl_3$) δ 1.45 (9H,s), 3.76 (6H, s), 6.69 (2H, s), 6.90 (1H, s), 7.34 (4H, m), 7.46 (2H, m), 7.90 (2H, d, J=7.25 Hz), 8.03 (2H, d, J=7.16 Hz); ^{13}C NMR ($CDCl_3$) δ 14.6, 23.1, 28.0, 29.7, 30.1, 32.3, 56.6, 78.0, 83.4, 96.5, 106.0, 125.7, 128.6, 128.7, 129.1, 129.2, 129.4, 129.8, 130.4, 132.0, 133.6, 13.9, 135.2, 151.3, 153.2, 166.1, 193.6

vi. 1-(3,5-Dimethoxy-4-nonayloxyl)-2-oxo-2-phenylethyl benzoate 51f:

MW: 532.62 yellow oil

^{1}H NMR ($CDCl_3$) δ 0.79 (3H, t, J=6.49 Hz), 1.20 (8H,m), 1.34 (2H, m), 1.63 (2H, t, J=7.5 Hz), 2.47 (2H, t, J=7.36 Hz), 3.80 (6H, s), 6.69 (2H,s), 6.90 (1H, s), 7.31 (4H, m), 7.44 (2H,m), 7.90 (2H, d, J=7.47 Hz), 8.02 (2H,d, J=7.42 Hz); ^{13}C NMR ($CDCl_3$) 19.5, 19.7, 17.0, 25.1, 27.5, 31.5, 31.7, 31.8, 34.3, 36.2, 58.5, 80.1, 98.6, 107.9, 108.2, 128.8, 129.3, 130.8, 131.2, 131.4, 131.9, 132.5, 134.4, 135.7, 135.9, 137.3, 154.8, 155.1, 155.3, 168.1, 172.9, 173.2, 195.7

vii. 1-(3,5-Dimethoxy-4-phosphanate)-2-oxo-2-phenylethyl benzoate 51g:

MW: 596.648 white solid

^{1}H NMR ($CDCl_3$) δ 1.30 (6H, t, J=6.49 Hz), 3.79 (6H,s), 4.20 (4H, dt, J=0.99, 3.33 Hz), 6.68 (1H,s), 6.88 (1H,s), 7.36 (4H, dd, J= 1.02, 7.66 Hz), 7.47 (2H,m), 7.90 (2H, d, J=7.27 Hz), 8.03 (2H, d, J=7.08 Hz); ^{13}C NMR ($CDCl_3$) δ 16.4, 16.5, 56.5, 56.6, 64.4, 64.5, 64.6, 64.7, 79.9, 78.3, 96.5, 106.2, 128.7, 128.9, 129.1, 129.2, 129.6, 129.8, 130.8, 130.9, 133.8, 152.3, 166.0, 193.5

3.1.3 General Procedure for the Photorelese Reaction of Benzoyl Benzoins

0.1 mmol of benzoyl benzoin was dissolved in 5 ml acetonitrile in a quartz tube ant the tube was placed in the Rayonet Reactor with four UV lights. The conversion of benzoyl benzoins to corresponding benzofuran derivatives were monitored with TLC. The products were purified with column chromatography with 1:3 EtOAc:Hexane.

i. 4,6-Dimethoxy-2-phenylbenzofuran 52a:

MW: 254.281 white solid

^{1}H NMR ($CDCl_3$) δ: 3.78 (3H,s), 3.95 (3H,s), 6.42 (1H, s) 6.50 (1H,s), 6.87 (1H, s), 7.28 (1H, t, J=7.39 Hz) 7.30 (2H, t, J=7.78 Hz), 7.75(2H, d, J=7.31 Hz);

ii. 4,6-Dimethoxy-2- phenylbenzofuran-5-yl acetate 52d:

MW: 428.518 white solid

^{1}H NMR ($CDCl_3$) δ 2.31 (3H, s), 3.78 (3H,s), 4.21 (3H,s), 6.70 (1H,s), 6.87 (1H, s), 7.27 (1H, t, J=7.39 Hz) 7.36 (2H, t, J=7.78 Hz), 7.73(2H, d, J=7.31 Hz); ^{13}C NMR ($CDCl_3$) 20.9, 56.9, 77.4, 96.5, 102.1, 125.2, 128.3, 129.0, 129.2, 130.6, 138.3, 140.7, 149.6, 157.0, 169.6

iii. 4,6-Dimethoxy-2-phenylbenzofuran-5-yl pivalate 52e:

MW: 354.396 white solid

^{1}H NMR ($CDCl_3$) δ: 1.51 (9H, s), 3.80 (3H, s), 4.21 (3H, s), 6.65 (1H, s), 6.83 (1H, s), 7.26 (1H, t, J=7.3 Hz), 7.34 (2H, t, J=7.4 Hz), 7.71 (2H, d, J=7.3 Hz); ^{13}C NMR ($CDCl_3$)

δ: 14.2, 27.7, 56.4, 60.4, 82.8, 96.1, 96.2, 101.6, 115.7, 124.7, 127.6, 128.5, 128.7, 129.6, 130.3, 138.1, 140.4, 149.6, 151.5, 156.5

iv. 4,6-Dimethoxy-2-phenylbenzofuran-5-yl nonanote 52f:

MW: 410.503 yellow oil

^{1}H NMR ($CDCl_3$) δ: 0.87 (3H, t, J=5.38 Hz), 1.44 (8H, m), 1.61 (2H,m), 1.79(2H, p, J=7.5 Hz), 2.58 (2H, t, J=7.4 Hz), 3.79 (3H, s), 4.21 (3H, s), 6.72 (1H, s), 6.92 (1H,s), 7.32 (1H,t, J=7.3 Hz), 7.40 (2H, t, J=7.4 Hz), 7.76 (2H, d, J=7.3 Hz) ^{13}C NMR ($CDCl_3$) δ: 14.58, 23.09, 25.55, 29.66, 29.74, 31.16, 32.28, 34.31, 56.67, 60.69, 77.00, 96.44, 96.57, 102.05, 125.24, 128.02, 128.86, 129.13, 129.41, 130.66, 138.34, 140.91, 149.74, 156.74, 171.88, 205.86

v. Diethyl 4,6-dimethoxy-2-phenylbenzofuran-5-yl phosphate 52g:

MW: 406.366 white solid

^{1}H NMR ($CDCl_3$) δ: 1.34 (6H, t, J=6 Hz), 3.83 (3H, s), 4.26 (3H,s), 4.26(4H, m), 6.66 (1H,s), 6.82 (1H,s), 7.27(1H, t, J=5.5 Hz), 7.35 (2H, t, J=3.5 Hz), 7.72 (2H, d, J=7.2 Hz); ^{13}C NMR ($CDCl_3$) δ:16.1, 16.2, 56.4, 60.5, 64.1, 64.2, 96.2, 96.4, 101.5, 124.7, 128.5, 128.7, 130.3

3.1.4 General procedure for the Hydrolysis of Protected Benzoins

1 mmol of benzoyl benzoin was dissolved in 50 ml acetonitrile and argon was bubbled through the solution for 15 minutes to remove the oxygen from medium. 1.5 equivalent NaOH in 50 ml H_2O and 35 ml CH_3CN was added dropwise for 2 hours. The resulting solution was stirred for an additional 2 hours at room temperature. The solution

was extracted with EtOAc and the organic phase was dried over $MgSO_4$. The product was obtained after purification with column chromatography 1:7 EtOac: hexane and 1:1 EtOAc:hexane in the case of phosphate derivative.

i. 2-Hydroxy-2-(3,5-dimethoxyphenyl)-1-phenylethanone 53a:

MW: 247.267 white solid

^{1}H NMR ($CDCl_3$) δ: 3.67 (6H, s), 4.37 (1H, d, J=5.9 Hz), 5.72 (1H, d, J=5.53 Hz), 6.22(1H, s), 6.35(2H,s), 7.30 (2H, t, J=7.8 Hz), 7.42 (1H, t, J=7.4 Hz), 7.83 (2H, d, J=7.3 Hz); ^{13}C NMR ($CDCl_3$) 55.5, 76.5, 96.6, 100.8, 106.1, 129.0, 129.5, 134.1, 134.2, 141.5, 161.6, 198.9

ii. 2-Hydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-1-phenylethanone 53b:

MW: 288.295 white solid

^{1}H NMR ($CDCl_3$) δ: 3.76 (6H, s), 4.32 (1H, broad), 5.38 (1H, broad), 5.72 (1H, s), 6.43 (2H, s), 7.34 (2H, t, J=6.94 Hz), 7.46 (1H, t, J=7.7 Hz), 7.83 (2H, d, J=7.1 Hz)

iii. 4-(1-Hydroxy-2-oxo-2-phenylethyl)-2,6-dimethoxyphenyl acetate 53c:

MW: 330.332 white solid

^{1}H NMR ($CDCl_3$) δ: 2.18 (3H, s), 3. 62 (6H, s), 4.40 (1H, broad s), 5.75 (1H,s), 6.45 (2H, s), 7.30 (4H, t, J=7.57 Hz), 7.42 (2H, t, J=7.45 Hz), 7.51 (2H, d, J=7.82 Hz); ^{13}C NMR ($CDCl_3$) 20.7, 56.4, 76.3, 96.5, 104.6, 129.1, 129.2, 129.5, 134.2, 134.3, 137.5, 152.9, 168.3, 199.0

iv. Tert-butyl 4-(1-hydroxy-2-oxo-2-phenyl ethyl)-2,6-dimethoxy phenyl carbonate 53d:

MW: 388.411

^{1}H NMR ($CDCl_3$) δ: 1.45 (9H, s), 3.70 (6H, s), 4.35 (1H, broad), 5.74 (1H, s), 6.45 (2H,s), 7.32 (2H, t, J=7.88 Hz), 7.45 (1H, t, J=7.41 Hz), 7.82 (2H, d, J=7.26 Hz)

v. 4-(1-Hydroxy-2-oxo-2-phenylethyl)-2,6-dimethoxyphenyl nonanoate 53e:

MW: 428.518 yellow oil

^{1}H NMR ($CDCl_3$) δ: 0.8 (3H, t, J=6.28 Hz), 1.21 (8H, broad s), 1.31 (2H, broad s), 1.69(2H, p, J=7.33 Hz), 2.53 (2H, t, J=6.59 Hz), 3.67 (6H, s), 4.43 (1H, broad), 5.80 (1H, s), 6.48 (2H, s), 7.35 (2H, t, J=7.79 Hz), 7.45 (1H, t, J=7.4 Hz), 7.86 (2H, d, J=8.48 Hz)

vi. Diethyl-4-(1-hydroxy-2-oxo-2-phenylethyl)-2,6-dimethoxyphenyl phosphate 53f:

white solid

^{1}H NMR ($CDCl_3$) δ: 1.27 (6H, dt, J=0.94 Hz, 3.75 Hz), 3.72 (6H,s), 4.17 (4H, dd, J=1.99 Hz, 3.22 Hz), 4.37 (1H, broad), 5.74(1H,s), 6.45 (2H,s), 7.34 (2H, t, J=7.8 Hz), 7.46 (1H, t, J=7.37 Hz), 7.86 (2H, d, J=7.32 Hz)

3.1.5 Synthesis of CPT benzoin Derivatives

Camptothecin was purchased from Boehringer Ingelheim KG.

i. 20-Benzoin camptothecin 55:

A suspension of camptothecin (0.4 mmol), 0.3 equivalent triphosgene and 3 equivalent DMAP in 20 ml anhydrous dichloromethane was stirred for 10 min. until the pink color was appeared. 1 equivalent of benzoin was added and the reaction mixture stirred for 12 hours. The solution was evaporated to dryness and the residual solid was purified by column chromatography 95:5 CH_2Cl_2:MeOH. MW: 586.59

^{1}H NMR (d-DMSO) δppm: 0.99 (3H, t, J=7.11 Hz), 2.26 (2H, q, J=6.31 Hz), 5.36 (2H, s), 5.56 (2H, s), 7.25 (1H, s), 7.43 (5H, m), 7.57 (1H, m), 7.64 (2H, d, J=6.24 Hz), 7.82 (1H, m), 7.99 (3H, m), 8.27 (1H, d, J=8.04 Hz), 8.37 (1H, d; J=8.32 Hz), 8.78 (1H, s); ^{13}C NMR (d-DMSO) 7.6, 30.7, 30.9, 50.5, 66.6, 78.3, 78.5, 80.4, 94.7, 95.0, 109.5, 111.1, 114.1, 119.3, 127.9, 128.2, 128.8, 129.0, 129.3, 129.9, 130.0, 130.7, 131.8, 132.7, 133.7, 134.2, 144.9, 146.4, 148.2, 152.5, 152.6, 156.6, 167.1, 193.0

ii. 20-(3',5'-Dimethoxy benzoin)- camptothecin 57a:

A suspension of camptothecin (0.5 mmol), 0.3 equivalent triphosgene and 3 equivalent DMAP in 20 ml anhydrous dichloromethane was stirred for 10 min. until the pink color was appeared. 1 equivalent of dimethoxybenzoin was added and the reaction mixture stirred for 12 hours. The solution was evaporated to dryness and the residual solid was purified by column chromatography 95:5 CH_2Cl_2:MeOH. MW: 390.39

^{1}H NMR (d-DMSO) δppm: 0.93 (3H, t, J=7.25 Hz), 2.18 (2H, q, J=6.31 Hz), 3. 68 (3H, s), 3.71 (3H, s), 5.26 (2H, s), 5.49 (2H, d, J=6.41), 6.43 (1H, s), 6.64 (2H, s), 7.01 (1H, s), 7.22 (1H, s), 7.29 (2H, t, J=7.58 Hz), 7.43 (1H, t, J=7.30 Hz), 7.65 (1H, t, J=7.30 Hz), 7.84 (3H, m), 8.04 (1H, d, J=8.01 Hz), 8.22 (1H, d; J=8.48 Hz), 8.59 (1H, s);^{13}C NMR (d-DMSO) 8.4, 11.6, 11.9, 24.8, 31.4, 51.1, 55.9, 56.2, 67.3, 76.5, 80.1, 81.0, 95.6, 100.0, 101.9, 106.1, 107.7, 115.8, 129.8, 129.9, 131.4, 134.1, 142.8, 145.5, 147.0, 148.9, 153.2, 157.2, 161.3, 161.6, 167.8, 193.5, 199.8

iii. 20-(3',5'-Dimethoxy-4'-acetoxybenzoin)- camptothecin 57b:

A suspension of camptothecin (0.4 mmol), 0.3 equivalent triphosgene and 3 equivalent DMAP in 20 ml anhydrous dichloromethane was stirred for 10 min. until the pink color was appeared. 1 equivalent of acetoxybenzoin was added ant the reaction mixture stirred for 12 hours. The solution was evaporated to dryness and the residual solid was purified by column chromatography 95:5 CH_2Cl_2:MeOH. MW: 704.67

^{1}H NMR (d-DMSO) δppm: 0.94 (3H, t, J=7.51 Hz), 2.19 (5H, m), 3. 76 (6H, s), 5.25 (2H, s), 5.50 (2H, d, J=4.5 Hz), 6.97 (2H, s), 7.10 (1H, s), 7.29 (1H, s), 7.37 (2H, t, J=7.87 Hz), 7.57 (1H, t, J=7.40 Hz), 7.74 (1H, t, J=7.88 Hz), 7.94 (1H, t, J=8.64 Hz), 7.96 (2H, d, J=8.23 Hz), 8.12 (1H, d; J=8.18 Hz), 8.30 (1H, s), 8.68 (1H, s).

iv. 20-(3',5'-Dimethoxy-4'-tert-butoxy benzoin)- camptothecin 57c:

A suspension of camptothecin (0.4 mmol), 0.3 equivalent triphosgene and 3 equivalent DMAP in 15 ml anhydrous dichloromethane was stirred for 10 min. until the pink color was appeared. 1 equivalent of t-Boc benzoin in 5 ml of anhydrous dichloromethane was added and the reaction mixture stirred for 12 hours. The solution was evaporated to dryness and the residual solid was purified by column chromatography 95:5 CH_2Cl_2:MeOH. MW: 720.72

^{1}H NMR (d-DMSO) δppm: 0.92 (3H, t, J=7.93 Hz), 1.43 (9H, s), 3. 70 (3H, s), 3.77 (3H, s), 5.23 (2H, s), 5.52 (2H, d, J=6.02 Hz), 5.75 (1H, s), 6.93 (1H, s), 6.98 (1H, s), 7.31-7.62 (3H, m), 7.74 (2H, q, J=7.12 Hz), 7.95 (3H, m), 8.14 (1H, d, J=8.23 Hz), 8.30 (1H, d; J=8.51 Hz), 8.72 (1H, s).

v. 20-(3',5'-Dimethoxy-4'-nonanoyloxy benzoin)- camptothecin 57d:

A suspension of camptothecin (0.4 mmol), 0.3 equivalent triphosgene and 3 equivalent DMAP in 15 ml anhydrous dichloromethane was stirred for 10 min. until the pink color

was appeared. 1 equivalent of nonanoylbenzoin in 5 ml dichloromethane was added and the reaction mixture stirred for 12 hours. The solution was evaporated to dryness and the residual solid was purified by column chromatography 95:5 CH_2Cl_2:MeOH. MW: 689.68

^{1}H NMR (d-DMSO) δppm: 0.94 (3H, t, J=7.51 Hz), 1.13 (3H, t, J=4.76), 1.37 (8H, broad s), 1.70 (2H, m), 2.34 (4H, m), 2.68 (2H, t, J=7.45 Hz), 3.80 (3H, s), 3.82 (3H, s), 5.38 (2H, s), 5,64 (2H, d, J=4.86 Hz), 6.94 (2H, s), 7.06 (1H, s), 7.11 (1H, s), 7.35 (2H, s), 7.48 (1H, t, J=7.40 Hz), 7.71 (1H, t, J=7.80 Hz), 7.91 (1H, t, J=8.37 Hz), 7.96 (2H, d, J=8.01 Hz), 8.15 (1H, d; J=8.22 Hz), 8.45 (1H, s), 8.80 (1H, s).

3.2 Modification on the C-7 of camptothecin

i. 7-Chloro methyl camptothecin 58:

To a suspension of camptothecin (0.1 gr) in a mixture of chloroacetaldehyde (0.6 ml) and H_2O (2.5 ml), 75% H_2SO_4 (2.5 ml) was added dropwise and then $FeSO_4.7H_2O$ (0.08 gr) was added. To the ice cold mixture, 30% H_2O_2 (0.5 ml) was added dropwise for 2 hours with stirring. The mixture was stirred at room temperature for an additional 14 hours and then diluted with H_2O, extracted with $CHCl_3$, and the organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the solvent gave a brown oil which was purified using column chromatography $CHCl_3$:MeOH 98:2 MW: 396.82

^{1}H NMR (DMSO-d_6) δ: 0.89 (3H, t), 1.88 (2H, q), 4.27 (2H, s), 5.40 (2H, s), 5.80 (2H, s), 6.50 (1H, s), 7.33 (1H, s), 7.60-8.21 (4H, m).

ii. 20-Acetyl camptothecin 60:

The solution of 2 ml trichloroethane, 2 ml of concantrated H_2SO_4 were added 50 mgr CPT and 125 mg of $AgNO_3$. The reaction was stirred in an oil bath at 50°C for 24 hours. The the mixture was poured into 25 ml of ice water and extracted with $CHCl_3$. The

product was purified by column chromatograph with 98:2 CH_2Cl_2:MeOH eluent. MW: 390.39 gr/mol.

^{1}H NMR ($CDCl_3$) δ: 0.92 (3H, t *J* = 7 Hz), 2.04 (2H, m), 2.16 (3H, s), 5.21 (2H, s), 5.28 (1H, Apart of AB system, *J*=17Hz), 5.58 (1H, B part of teh AB system *J*= 17 Hz), 7.13 (1H, s), 7.19 (1H, s), 7.59 (1H, t, J= 7 Hz), 7.76 (1H, t, J= 7 Hz), 7.85-8.15 (2H, m), 8.31 (1H,s).

iii. Trimethylsilylamine camptothecin 61:

7-chloromethyl camptothecin (68 mg) in 4 ml dry DMSO was allowed to stir untill all the solid was dissolved. To the remainig solution was added a solution of 0.02 ml trimethylsilyl methylamine in 4 ml dry toluene. The reaction mixture was allowed tos tir for 2 hours. The product was purified as a yellow solid by column chromatography eluted with $CHCl_3$:MeOH 95:5. MW: 433.53

^{1}H NMR(DMSO-d_6)δ:0.89 (3H,t *J*=7 Hz), 1.88 (2H, q, *J*=4 Hz),5.27 (2H, d, *J*= 4Hz), 5.40 (2H,s), 5.80 (2H, s), 5.80 (1H,t, *J*=4Hz), 6.50 (1H, s), 7.33 (1H, s), 7.60-8.21 (4H,m).

iv. 7-Hydroxyl methyl camptothecin 62:

To a suspension of camptothecin (0.3 gr) in a mixture of MeOH (9ml) and H_2O (7.5 ml), 75% H_2SO_4 (7.5 ml) was added dropwise and then $FeSO_4.7H_2O$ (0.24 gr) was added. To the ice cold mixture, 30% H_2O_2 (1.5 ml) was added dropwise for 1.5 hours with stirring. The mixture was stirred at room temperature for an additional 14 hours and then diluted with H_2O. The precipitate was collected on a celite pad by suction. The pad was eluted with hot DMF and the eluent was evaporated to dryness. MW: 378.38

^{1}H NMR (DMSO-d_6) δ: 0.89 (3H,t *J*=7 Hz), 1.88 (2H, q, *J*=4 Hz),5.27 (2H, d, *J*= 4Hz), 5.40 (2H,s), 5.80 (2H, s), 5.80 (1H,t, *J*=4Hz), 6.50 (1H, s), 7.33 (1H, s), 7.60-8.21 (4H,m).

v. 7-Formylmethyl camptothecin 63:

A suspension of 7-hydroxymethyl camptothecin (117 mg) in acetic acid (50 ml) was heated under reflux for 5.5 hours. The mixture was evaporated to dryness and the residue was purified with column chromatography eluted with $CHCl_3$:MeOH 98:2. MW: 376.36

^{1}H NMR ($CDCl_3$) δ:1.18 (1H,d J= 8Hz), 1.93 (2H, q, *J*=8 Hz),5.31 (1H, d, *J*= 16Hz), 5.63 (2H,s), 5.80 (1H,d, *J*=16Hz), 7.68 (1H, s), 7.85-7.95 (2H, m), 8.35-8.41 (1H,m), 8.77-8.83 (1H,m), 11.20 (1H,s).

vi. 7-Imino-trimethylsilyl camptothecin 64:

To a suspension of $Yb(OTf)_3$ in 2.5 ml of anhydrous CH_2Cl_2 containing activated 4Å MS, a solution 0f 7-formylmethyl CPT in 10 ml of CH_2Cl_2 was added, followed by a solution of amine in 0.5 ml of CH_2Cl_2. The resulting mixture is stirred at room temperature for one day. After filtering the sieves, the solution was washed with water where the water phase was rapidly extracted with CH_2Cl_2. The crude product could not been purified enough.

vii. Mercapto-undecanoic acid-2,3-dioxo-pyrrolidin-1-yl ester 65:

The 11-mercaptoundecanoic acid (1.08 gr) was reacted with N-hydroxy succinimide (0.69 gr) was stirred in anhydrous THF in the presence of DCC (1.23 gr) under argon atmosphere. The solution was filtered of from celite pad and eluted with EtOAc. The eluent was evaporated to dryness.

viii. 20- Mercapto-undecanoic acid-2,3-dioxo-pyrrolidin-1-yl ester CPT 66:

A suspension of CPT (50 mg), Sc(OTf) (42.5 mg) and DMAP (50 mg) in anhdrous CH_2Cl_2 was cooled to -8°C in an ice-salt bath, followed by the addition of mercapto ester. The reaction mixture was stirred at -8°C for 30 minutes and allowed to warm to room temperature over 2 hours. The reaction mixture was then filtered and the filtrate was washed with 20 ml of 0.1 N HCl and 20 ml of 0.1 M $NaHCO_3$ and 20 ml of water. The evaporation of the solvent yielded the crude product.

ix. 7-Cyclohexyliminocamptothecin 68:

To a suspension of $Yb(OTf)_3$ (7 mg) in 2.5 ml of anhydrous CH_2Cl_2 containing activated 4Å MS, a solution of 7-formylmethyl CPT (39 mg) in 5 ml of CH_2Cl_2 was added, followed by a solution of the cyclohexylamine in 0.5 ml of CH_2Cl_2. The resulting mixture is stirred at room temperature for one day. After filtering the sieves, the solution was washed with water where the water phase was rapidly extracted with CH_2Cl_2.

x. 7-Cyclohexyliminocamptothecin-Pd complex 69:

The crude product of the above step was reacted with palladium acetate in the presence of sodium acetate in MeOH. The product could not be purified.

3.3 Synthesis of cisplatin derivatives

i. 2,3-Dihydroxy-succinic acid dimethyl ester 70:

Tartaric acid (23.6 gr) and methanol (30 ml) was stirred in 50 ml $CHCl_3$. Then 0.1 ml of HCl was added to the solution and refluxed rof 24 hours with a back Dean-Stark trap. The product was purified by vacuum distilattion and obtained at 0.2 mmHg, 100°C. The product was obtained as a colorless dense liquid. MW: 178

ii. 2-Phenyl-[1,3]dioxalane-4,5-dicarboxylic acid dimethyl ester 71:

Dimethyl tartarate (9.8 gr) and 5 ml benzaldehyde was dissolved in 120 ml benzene. A catalytic amount of pTsOH was added to the solution and then the solution heated to reflux for 5 days under Dean-Stark trap. The product was purified by distillation and obtained at 1mmHg, 120°C. The product was obtained as a white crystall. MW: 266.25
^{1}H NMR ($CDCl_3$) δ: 3.71 (3H, s), 3.80 (3H, s), 4.74 (1H, s), 4.88 (1H, s), 6.01 (1H, s), 7.30-7.48 (5H, m).

iii. 2-Phenyl-[1,3]dioxalane-4,5-dicarboxylic acid diamide 72:

NH_3 gas was passed over a solution of the diol protected dimethyl tartarate (4.5 gr) in 50 ml methanol for 15 minutes and then the solution was stirred for a night at 0°C. The solution was evaporated to dryness. MW: 236.22
^{1}H NMR ($CDCl_3$) δ: 4.72 (2H, s), 5.86 (1H, s), 6.08 (2H, d, J=48.83 Hz), 6.68 (2H, d, J=44.61 Hz), 7.30 (3H,broad s), 7.41 (2H, broad s)

iv. *cis*-(5-Aminoethyl-2-methy-2-phenyl-[1,3]-dioxalan-4-yl)-methylamine 73:

To an ice cooled suspension solution of 0.4 gr of $LiAlH_4$ in 20 ml dry ether was added in small portions to 0.4 gr of the product of above step. After the completition of the addition, the reaction mixture was heated at reflux for 3 hours. Ether was added and

the excess $LiAlH_4$ was cautiously decomposed by the addition of water. The solution was first extracted with 2 N HCl. Ammonium hydroxide was added on to the aqeous phase and extracted with ether. The organic phase is then collected and evaporated to dryness. The product was added as a yellow oil. MW: 209

v. 2-((Quinolin-4-yl)methylene)malonic acid 74:

To a suspension of 4-quinolinecarboxaldehyde (0.1 gr) and iodine (0.12 gr) in 20 ml ethanol was added malonic acid (0.1 gr). The mixture was stirred in an ice bath and 0.1 ml of piperideine was added to the solution. Then the mixture was heated under reflux for 5 hours and stirred at room temperature for an additional 12 hours. The mixture was evaporated to dryness and the obtained yellow oily substance was recrystallized in $CHCl_3$. The white precipitate was collected by suction filtration. MW: 230.19

^1H NMR ($CDCl_3$) δ: 7.34 (1H, d, J=4.07 Hz), 7.63 (1H, t, J=6.70 Hz), 7.81 (1H, t, J=2.88 Hz), 7.82 (1H, d, J=7.47 Hz), 8.03 (1H, d, J=8.21 Hz), 8.20 (1H, s), 8.82 (1H, d, J=4.42 Hz)

vi. 2-((Quinolin-4-yl)methylene)malonic acid disilver salt 75:

The product of the above step was dissolved in 1 ml H_2O and 60µl %25 NH_3 solution. $AgNO_3$ solution (140 mg in 1 ml H_2O) was then added to the solution. The precipitate was filtered and washed with water to remove excess NO_3^-. The product was obtained as a yellow solid.

vii. 2-((Quinolin-4-yl)methylene)malonic acid-cisplatin complex 76:

The disilver salt product of the above step (15 mg) and cisplatin (10 mg) in 10 ml H_2O was heated to 60°C for 16 hours. The mixture was then extracted with $CHCl_3$ and

the collected organic phase was evaporated to dryness but no trace of product was obtained.

viii. 2-(7 –Ethylene-camptothecin) malonic acid 77:

To a suspension of 7-formylmethyl camptothecin (10 mg) and malonic acid (4 mg) in 5 ml ethanol, iodine (5 mg) was added with stirring. The solution was placed in a oil bath at 50°C and a trace amount of piperidine was added to the solution. The precipitate obtained during the reaction was filtered. MW: 462.41

^{1}H NMR ($CDCl_3$) δ: 1.01 (3H, t, J=7.57 Hz), 1.94 (2H, q, J=6.82 Hz), 5.44 (2H, s), 5.49 (2H,s), 6.52 (1H, s) 6.59 (1H,s), 7.42 (1H, s), 7.82 (1H, t, J= 6.95 Hz), 7.97 (1H, t, J=7.2 Hz), 8.27 (1H, d, J=8.16 Hz), 8.48 (1H, d, J=8.63 Hz)

CHAPTER 4

CONCLUSION

Many therapeutic drugs have undesirable properties that may become pharmacological, pharmaceutical, or pharmacokinetic barriers in clinical drug application. Among the various approaches to minimize the undesirable drug properties while retaining the desirable therapeutic activity, the chemical approach using drug derivatization offers perhaps the highest flexibility and has been demonstrated as an important means of improving drug efficacy. There are many efforts for the modification of camptothecin to obtain new derivatives addressing the solubility and stability problems. It was also the aim of this study to synthesize new camptothecin derivatives and prodrugs possesing high solubility and high stability but less toxicity.

In the first part of the study a targetted prodrug approach was modified on camptothecin drug. The 20-OH functional group of camptothecin was replaced with a benzoin derivative that is able to release the drug under photolysis at 350 nm. The new prodrugs should be more stable than camptothecin itself since the protection of 20-OH provides stabilization. The synthesis of the aimed prodrugs were achieved after several steps and the new prodrugs possesed higher stability as expected. The *in vitro* irradiation of the prodrugs at 350 nm was also succeded without any decomposition of the drug.

The former steps of the prodrug synthesis was also performed succesfully. The unsymmetrical benzoin derivatives substituted with both methoxy groups that are requisted for efficient photorelease and the representative groups necessary for controlled release were synthesized and used for derivatization. Besides, the kinetics of photoreleasing abilities of the new benzoin derivatives were examined and it was found

that the new derivatives have equal or more photoreleasing ability when compared with the reference benzoin derivative.

The prodrugs are expected to be inert Prodrugs can be viewed as drugs containing specialized non-toxic protective groups used in a transient manner to alter or to eliminate undesirable properties in the parent molecule. The new prodrugs are now being investigated by means of biological activity and toxicity and preliminary results showed that they are non toxic. As a result, new camptothecin prodrugs with higher stability in buffered solution and higher solubility due to the solubility properties of the substituents, able to carry the lactone form of the drug to the targetted places.

The second part of the study comprises the earlier studies on the modification of camptothecin. It is well known that the 7^{th} position of camptothecin is the most suitable place for the modification. In this part of the study 7-amino and silyl substituted camptothecins were synthesized. The most important drawback of the camptothecin experiments is to remove the unreacted camptothecin from the new compound. The targetted derivatives were synthesized as pure as possible.

Combination of camptothecin with some other drugs such as cisplatin was also investigated in this study. The synthetic efforts showed that the reactions are very promising and the combination studies can be studied as a major subject in the future.

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APPENDIX A: Spectroscopic Data

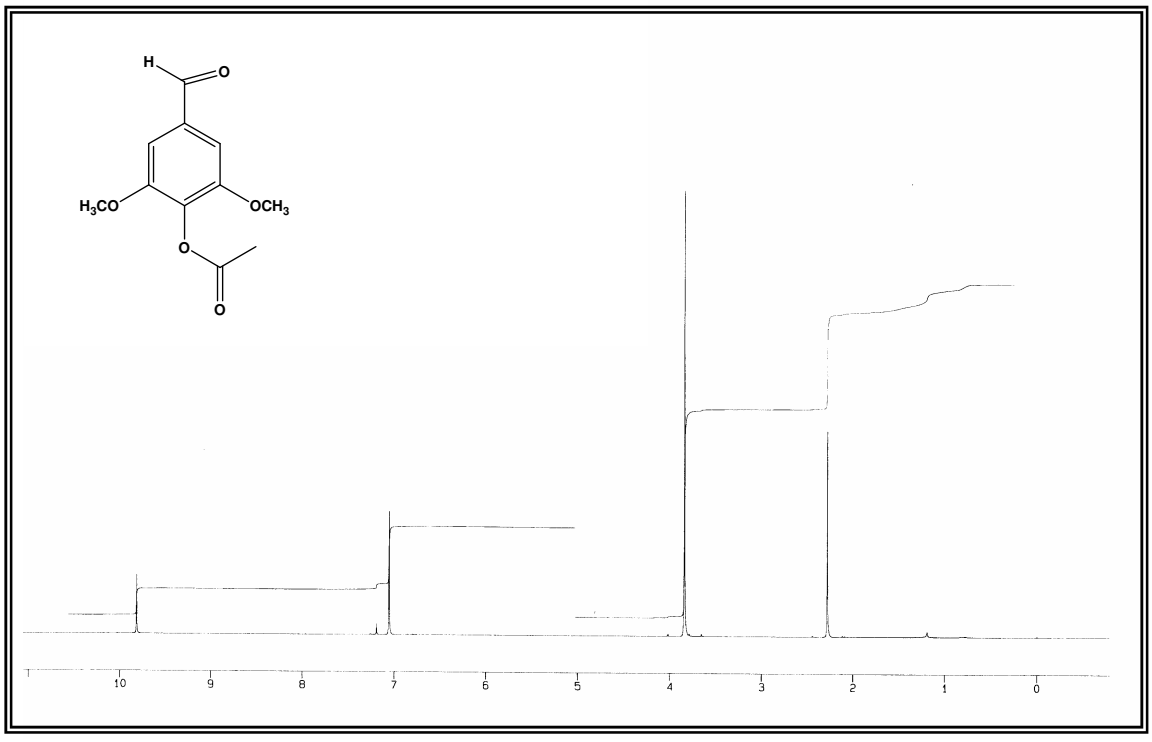


Figure 29: ^{1}H NMR spectrum of (48a)

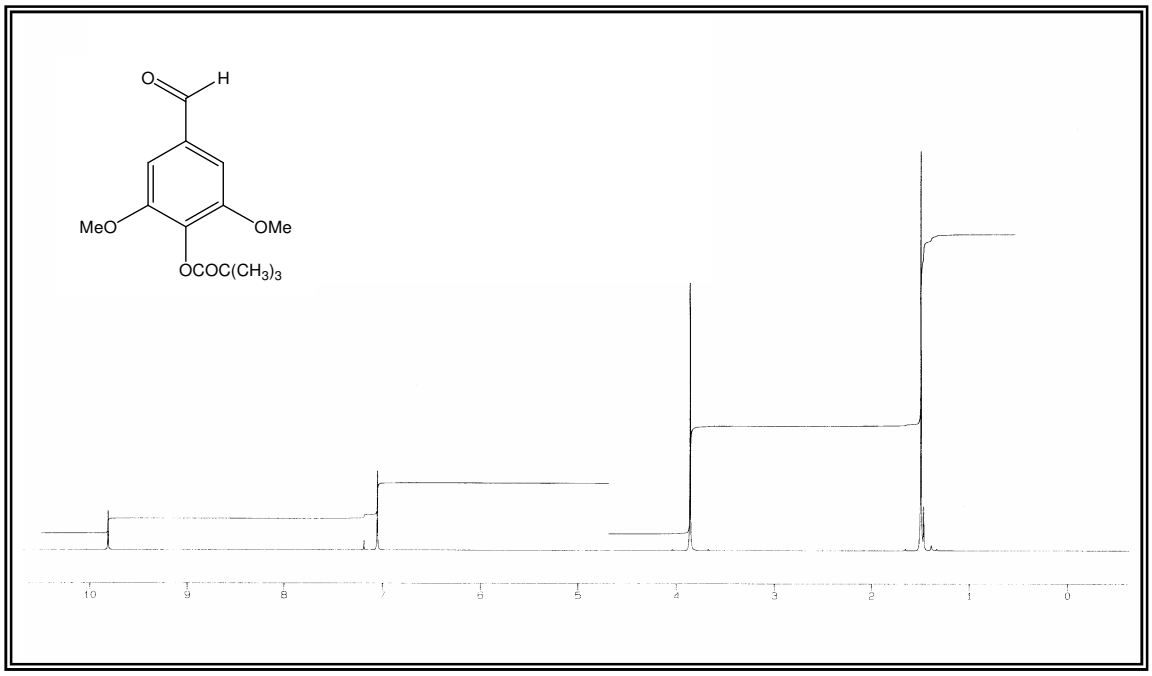


Figure 30: ^{1}H NMR spectrum of (48)

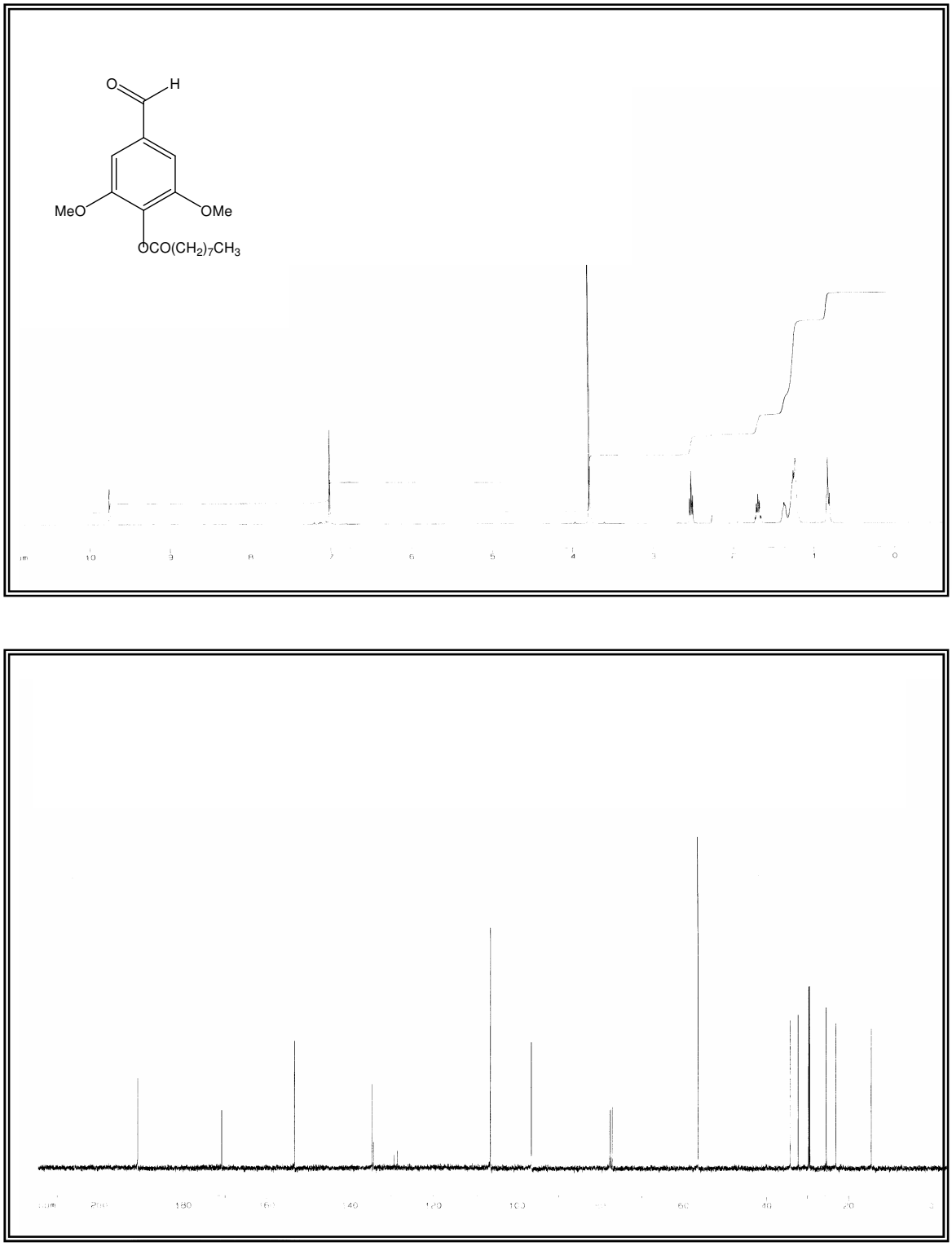


Figure 31: ^{1}H and ^{13}C NMR spectra of (48c)

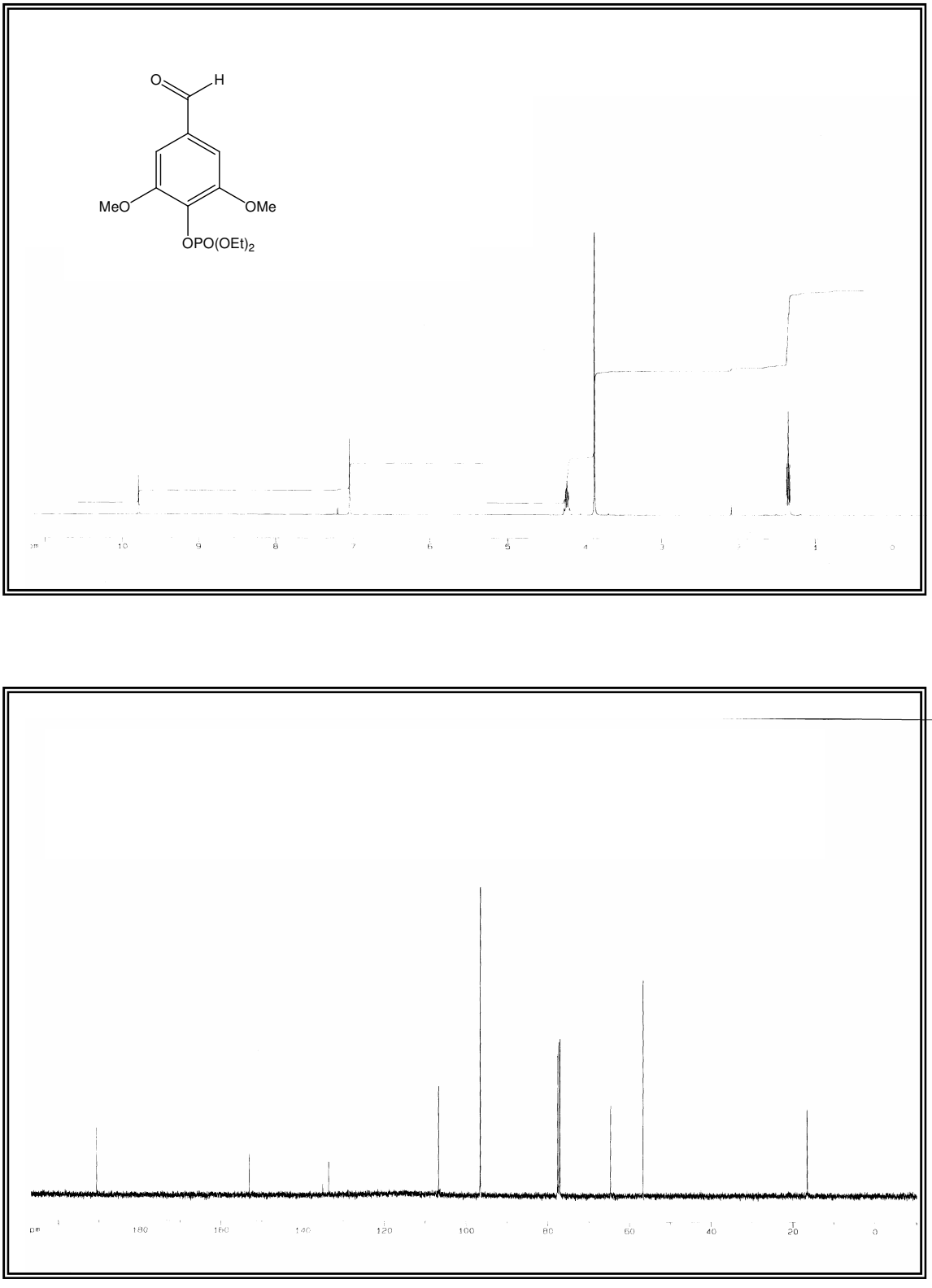
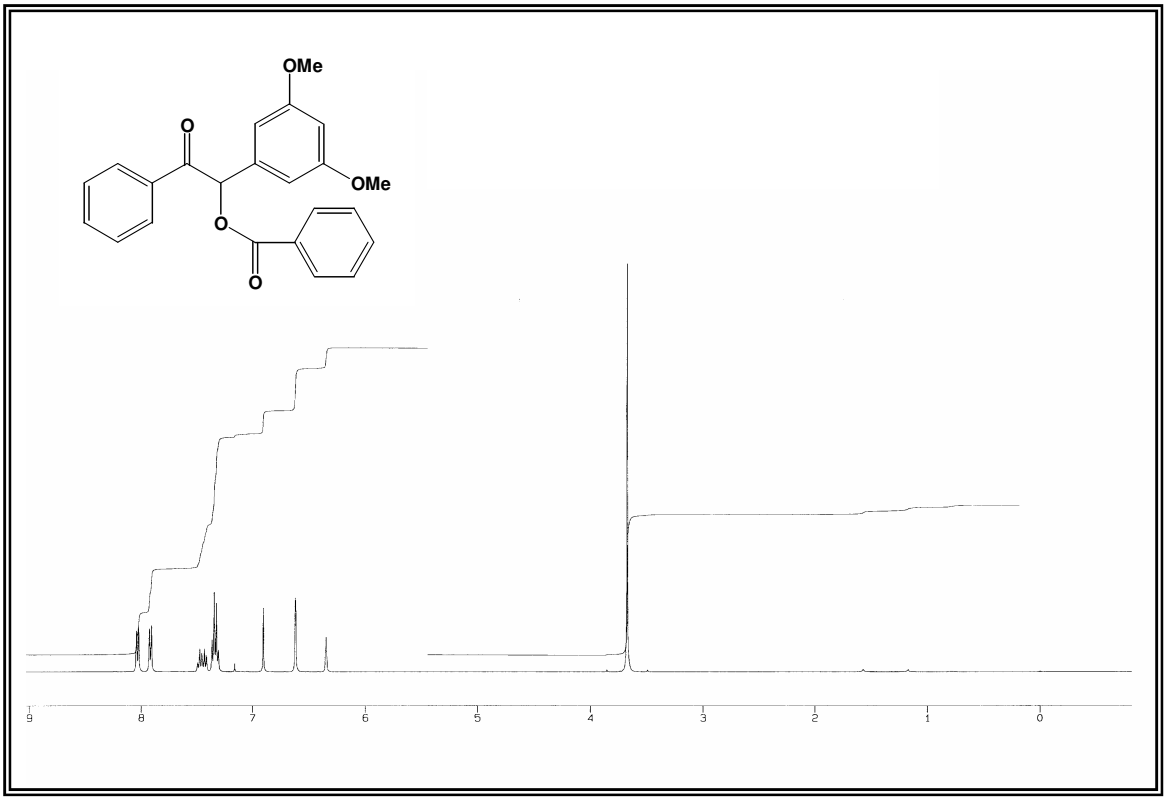


Figure 32: ^{1}H and ^{13}C NMR spectra of (48d)



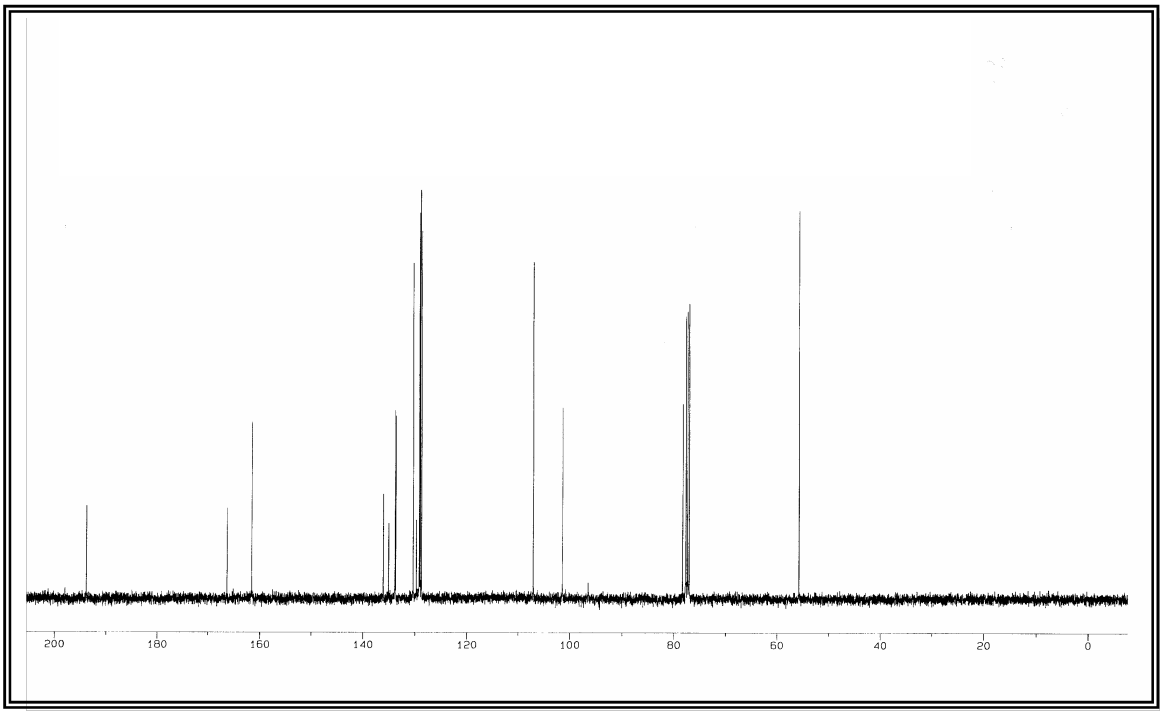


Figure 33: ^{1}H NMR spectrum of (51a)

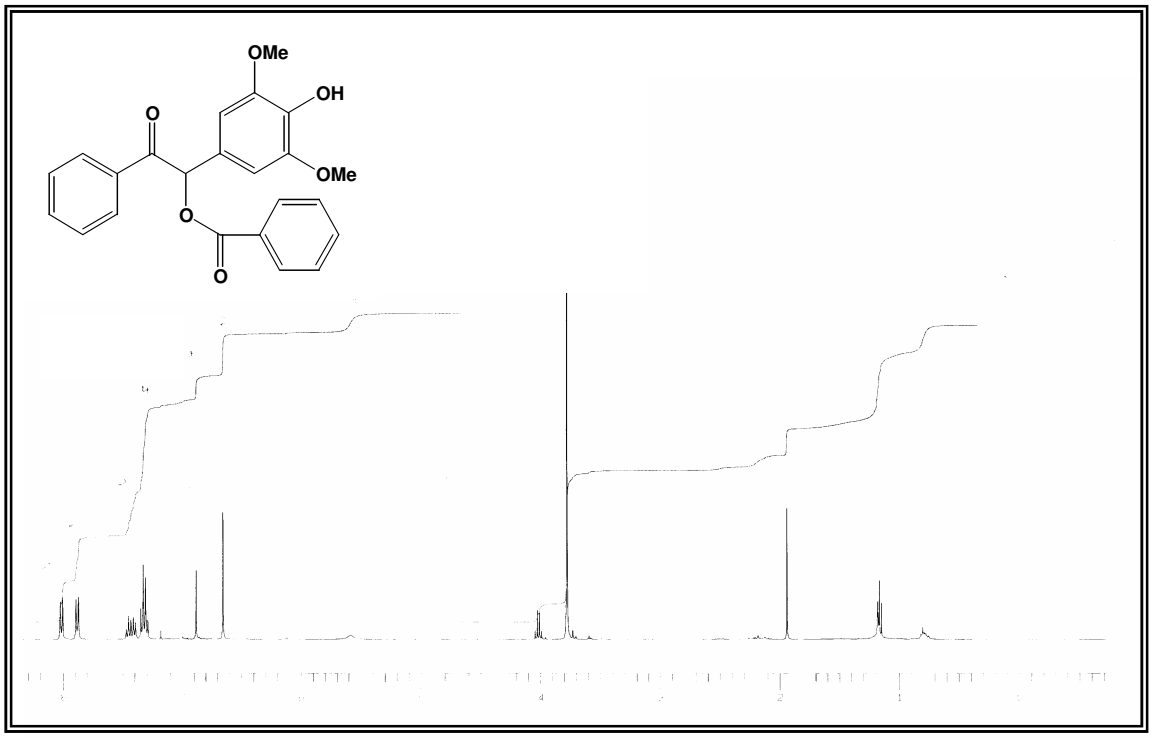


Figure 34: ^{1}H NMR spectrum of (51b)

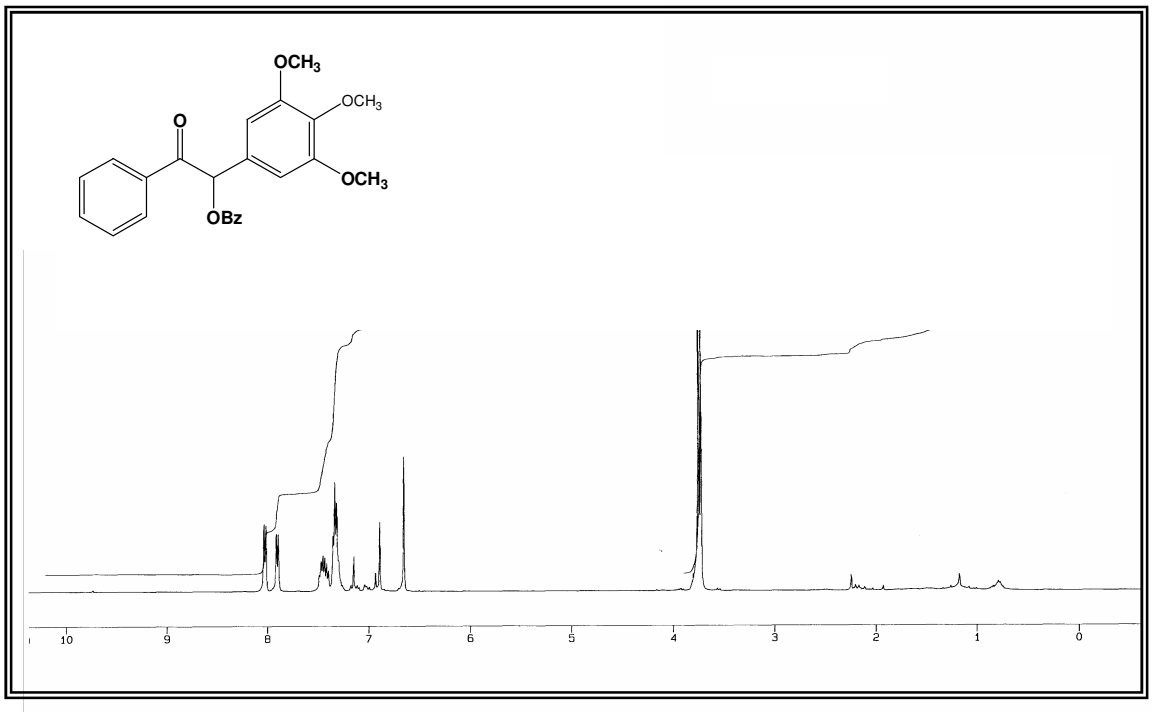
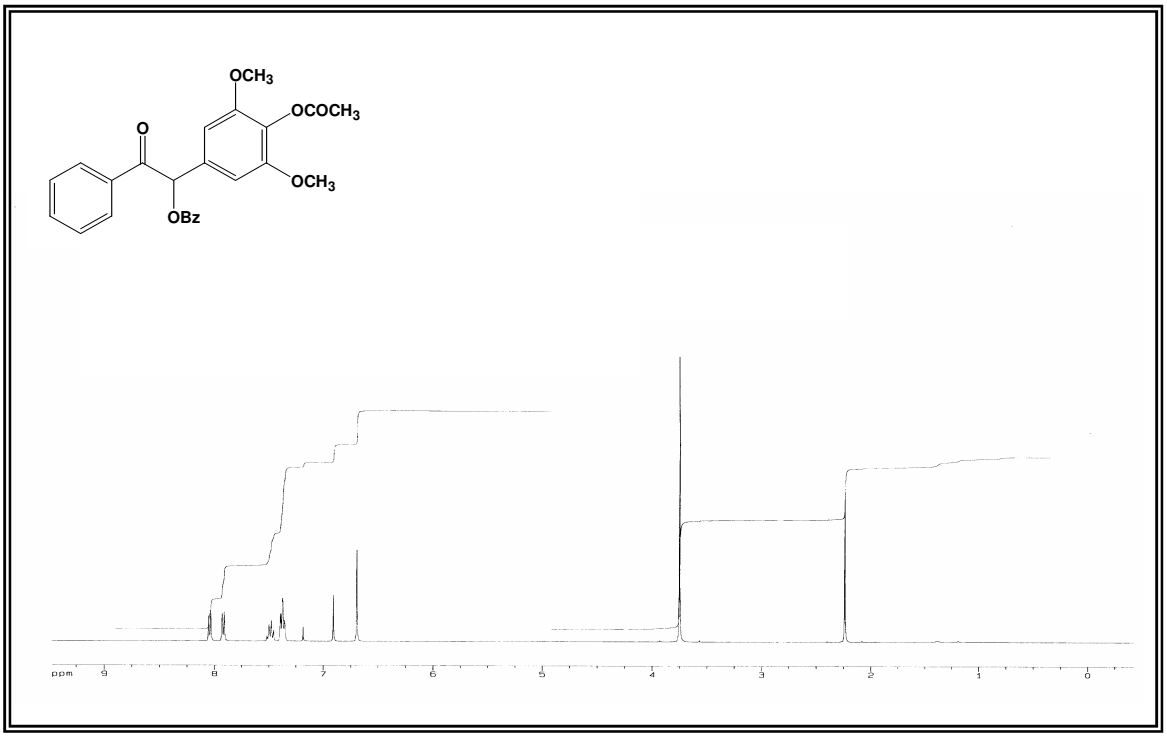


Figure 35: ^{1}H NMR spectrum of (51c)



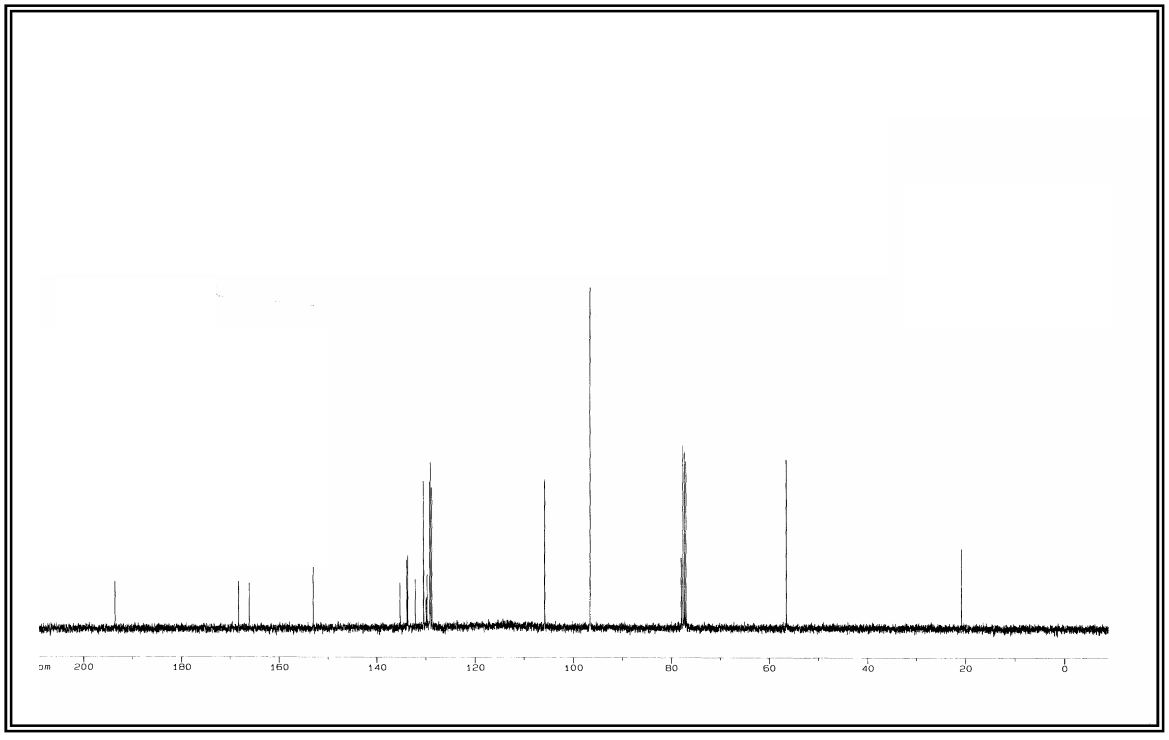


Figure 36: ^{1}H and ^{13}C NMR spectra of (51d)

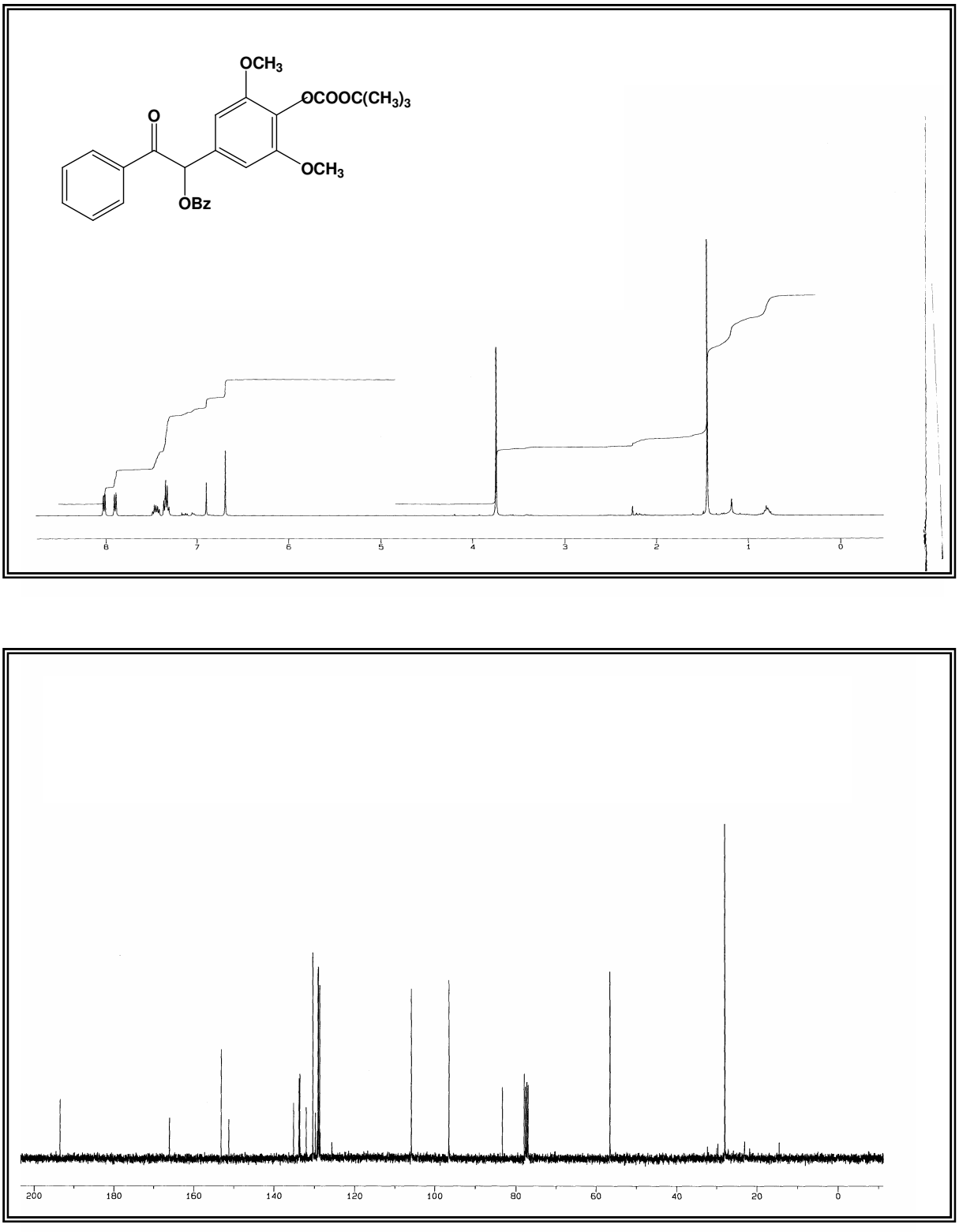


Figure 37: ^{1}H and ^{13}C NMR spectra of (51e)

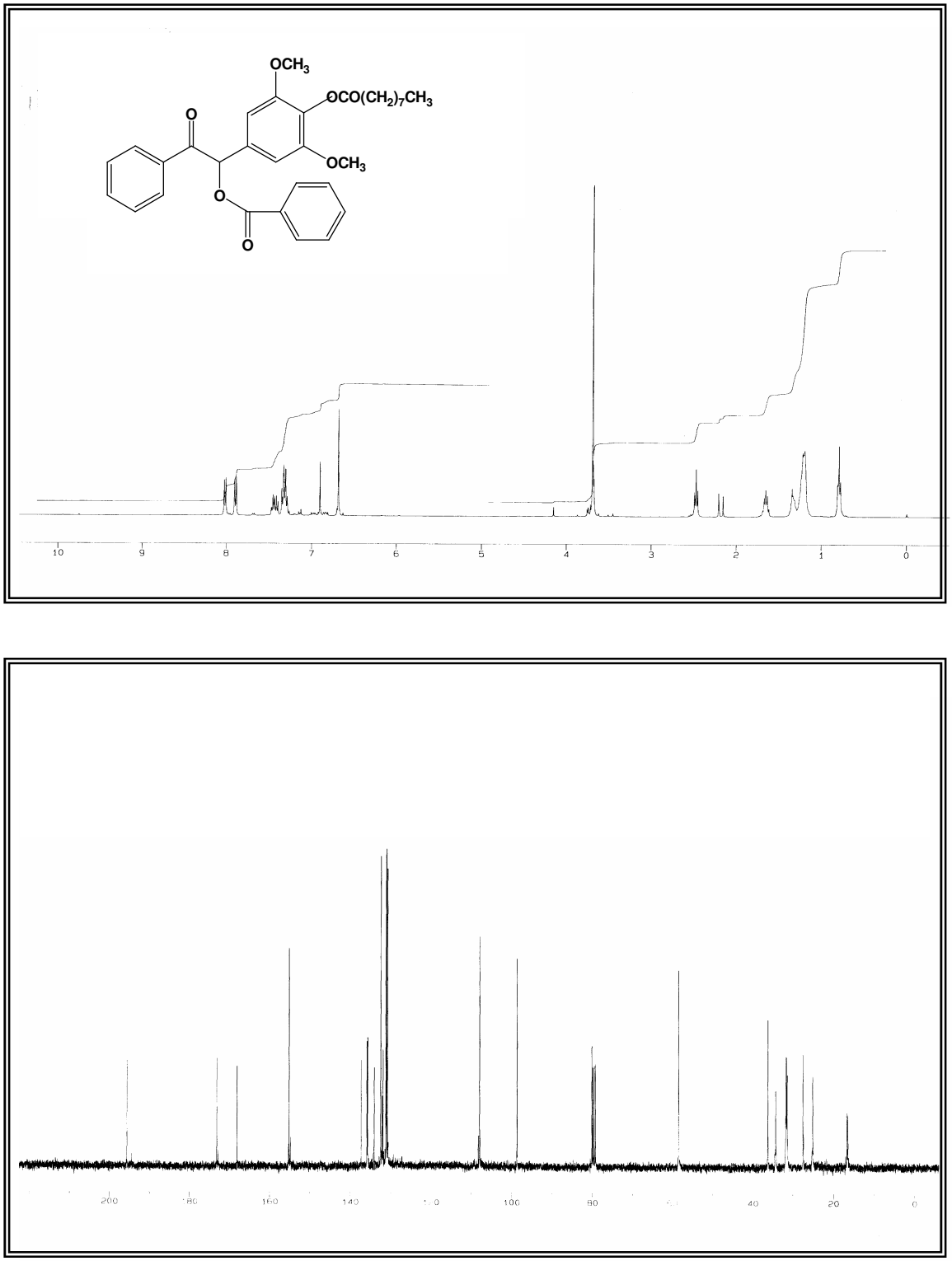


Figure 38: ^{1}H and ^{13}C NMR spectra of (51f)

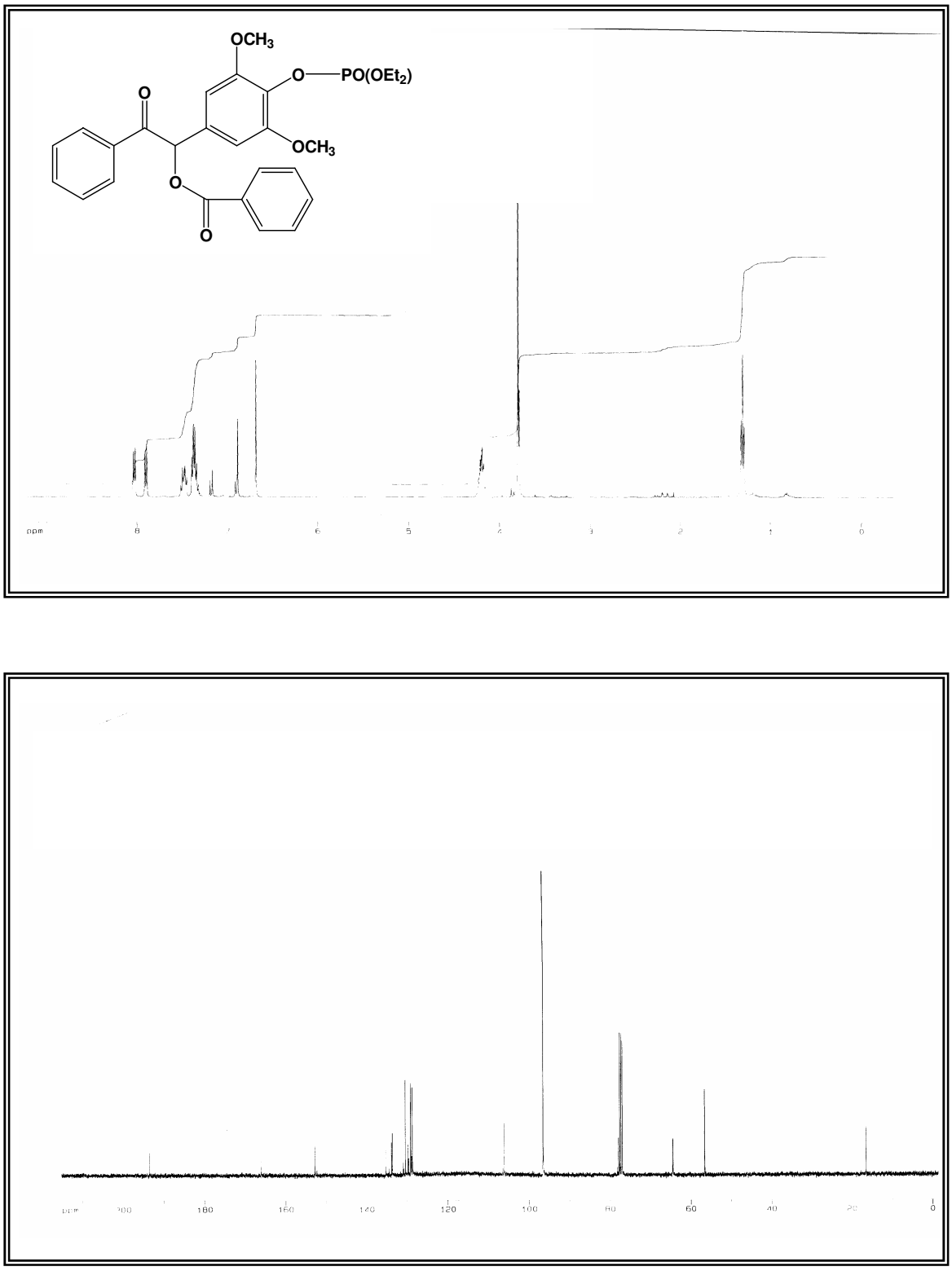


Figure 39: ^{1}H and ^{13}C NMR spectra of (51g)

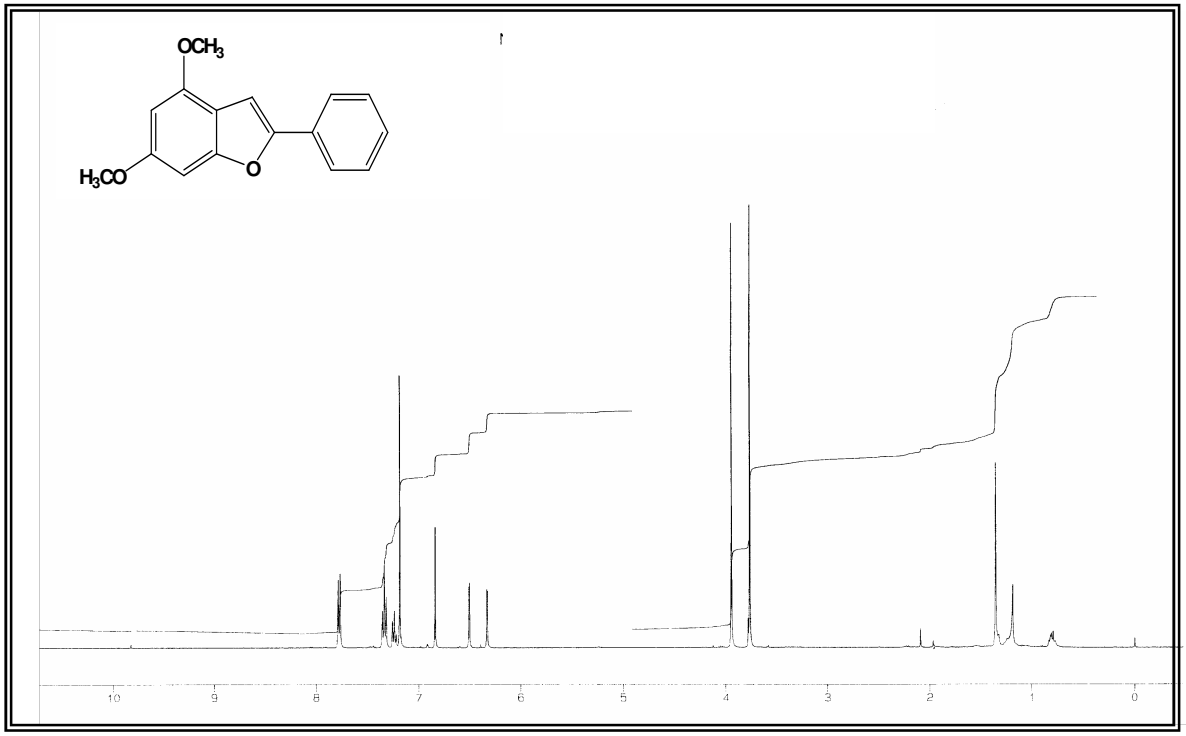


Figure 40: ^{1}H NMR spectrum of (52a)

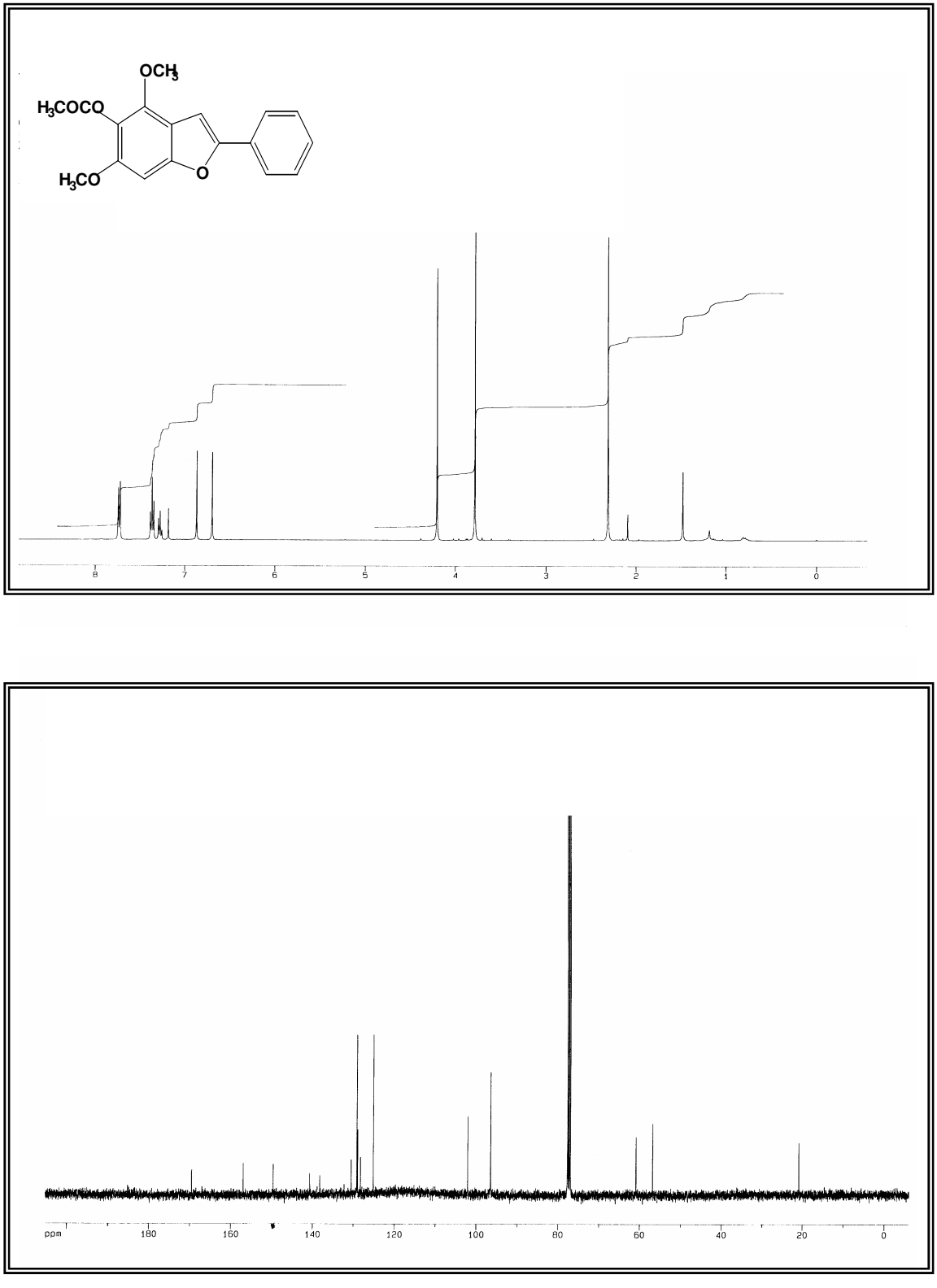
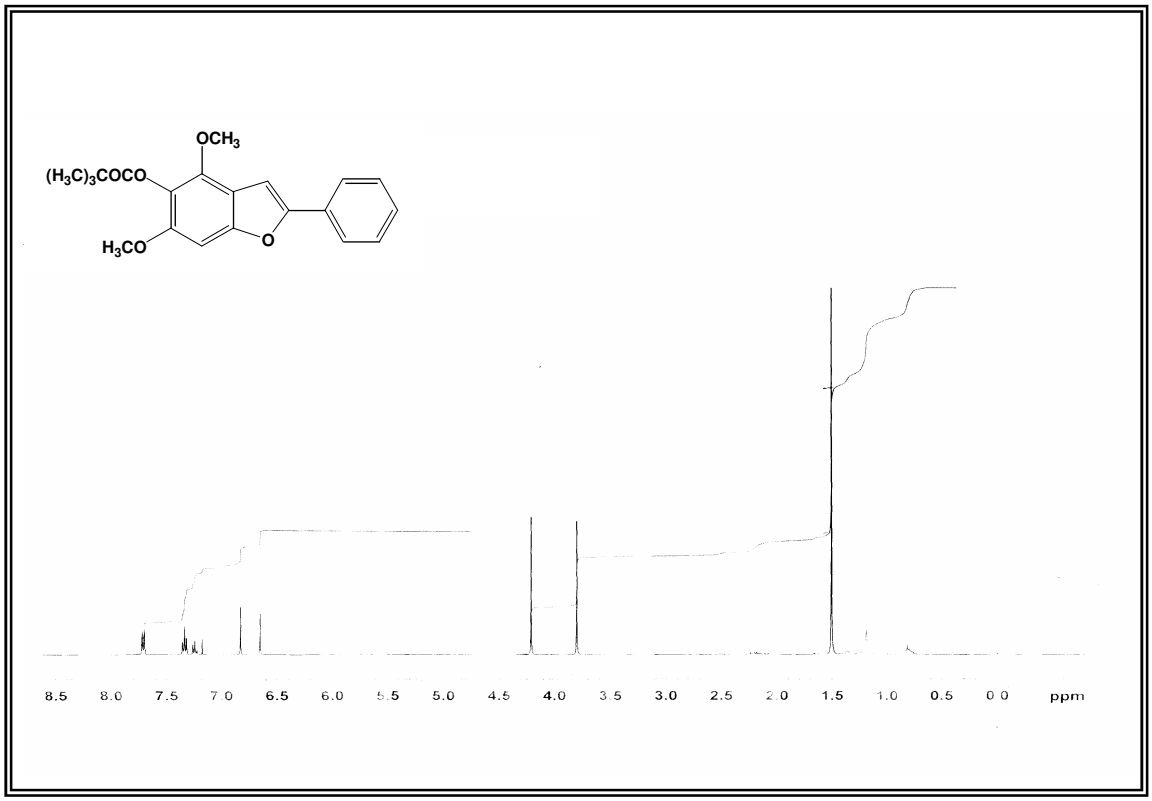


Figure 41: ^{1}H and ^{13}C NMR spectra of (52d)



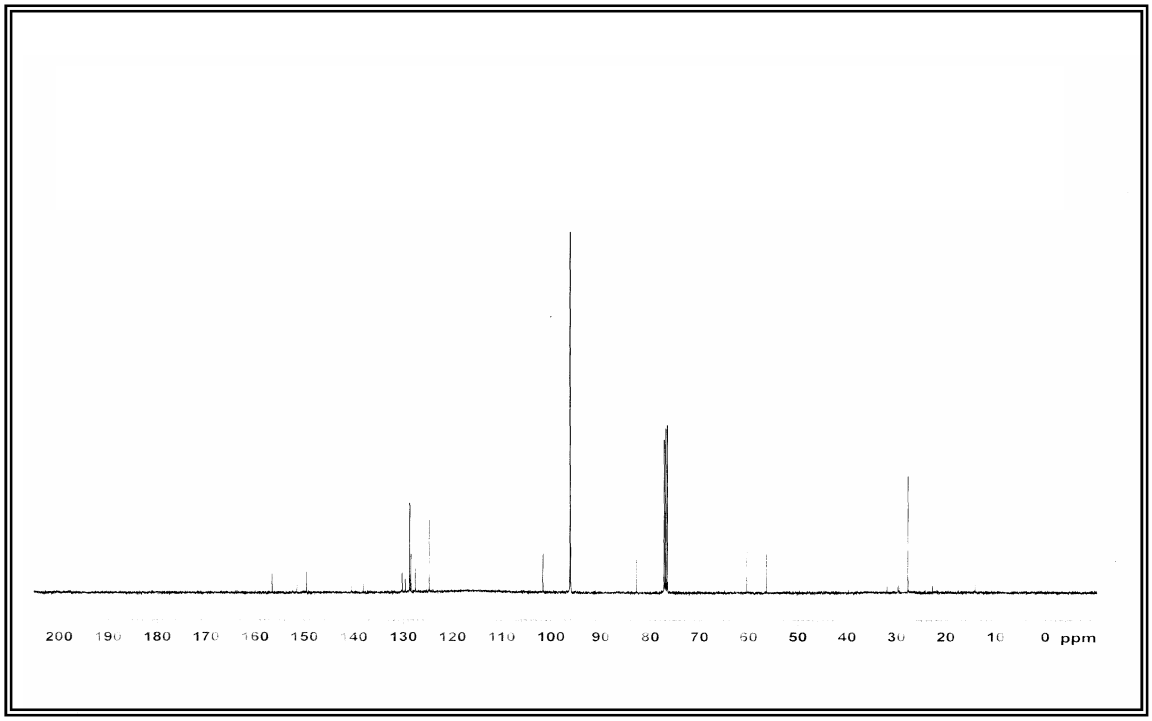


Figure 42: ^{1}H and ^{13}C NMR spectra of (52e)

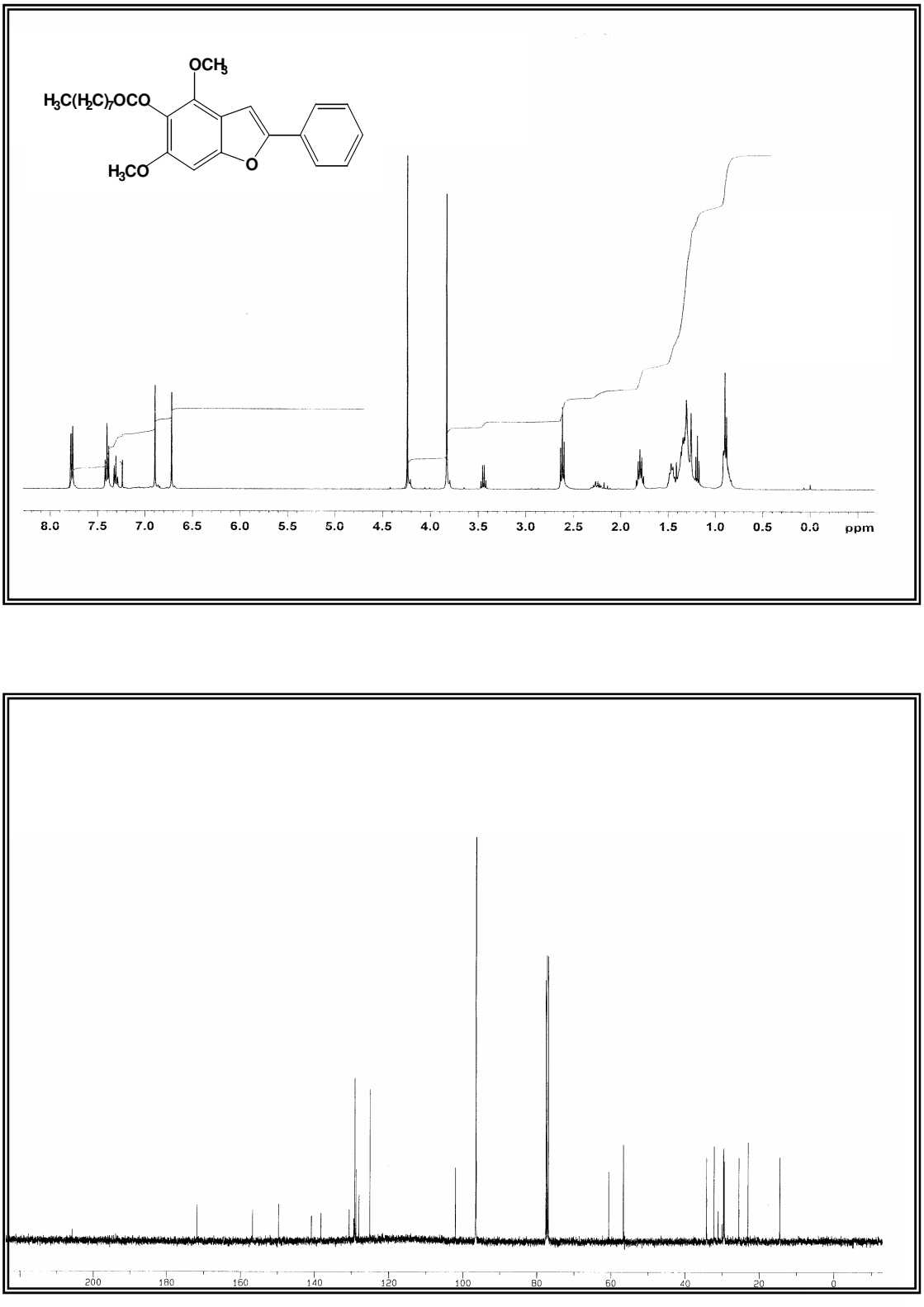
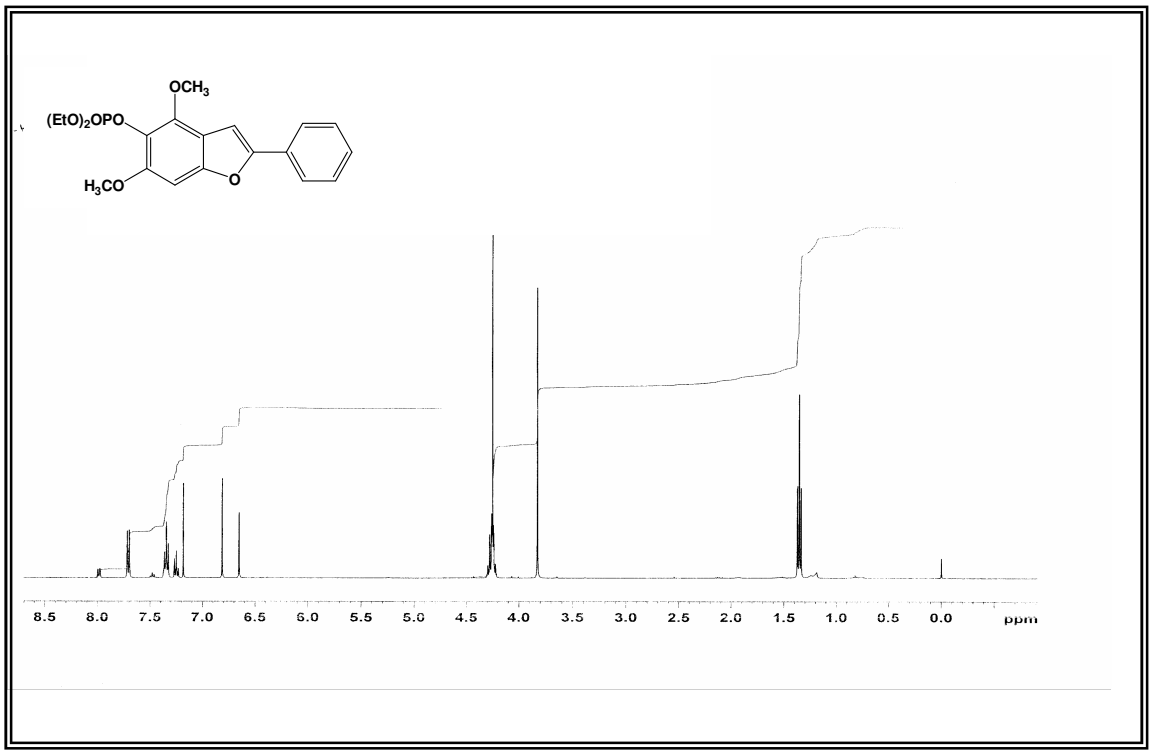


Figure 43: ^{1}H and ^{13}C NMR spectra of (52f)



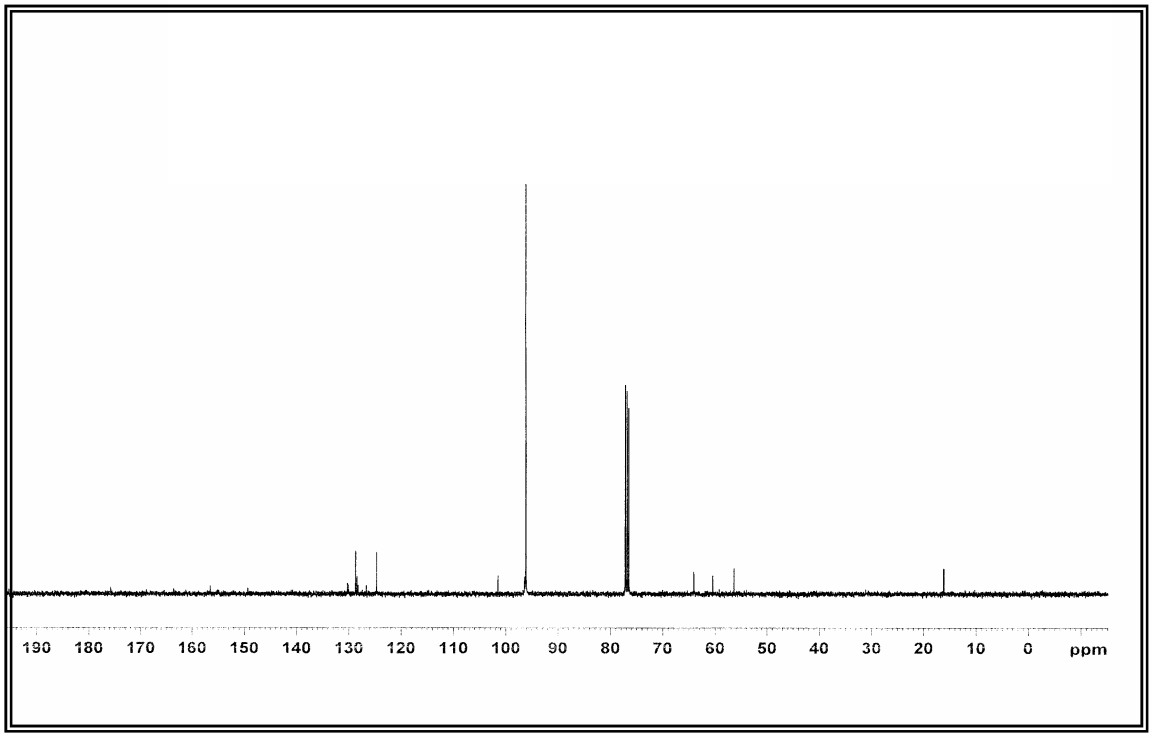
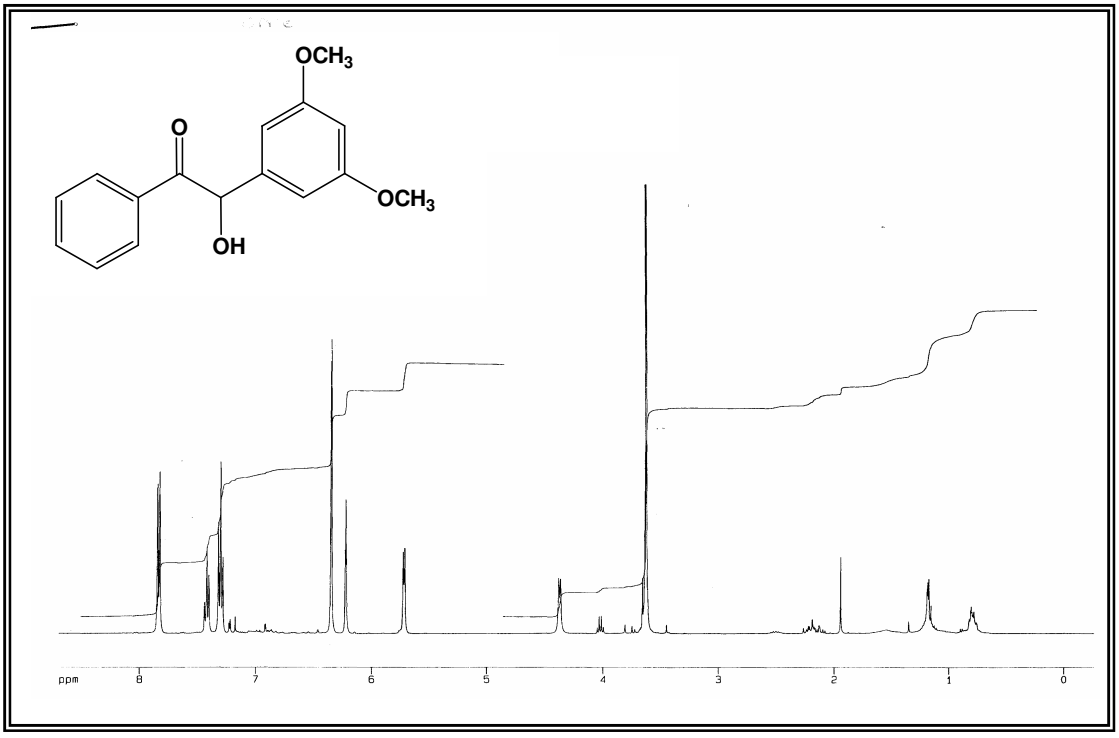


Figure 44: ^{1}H and ^{13}C NMR spectra of (52g)



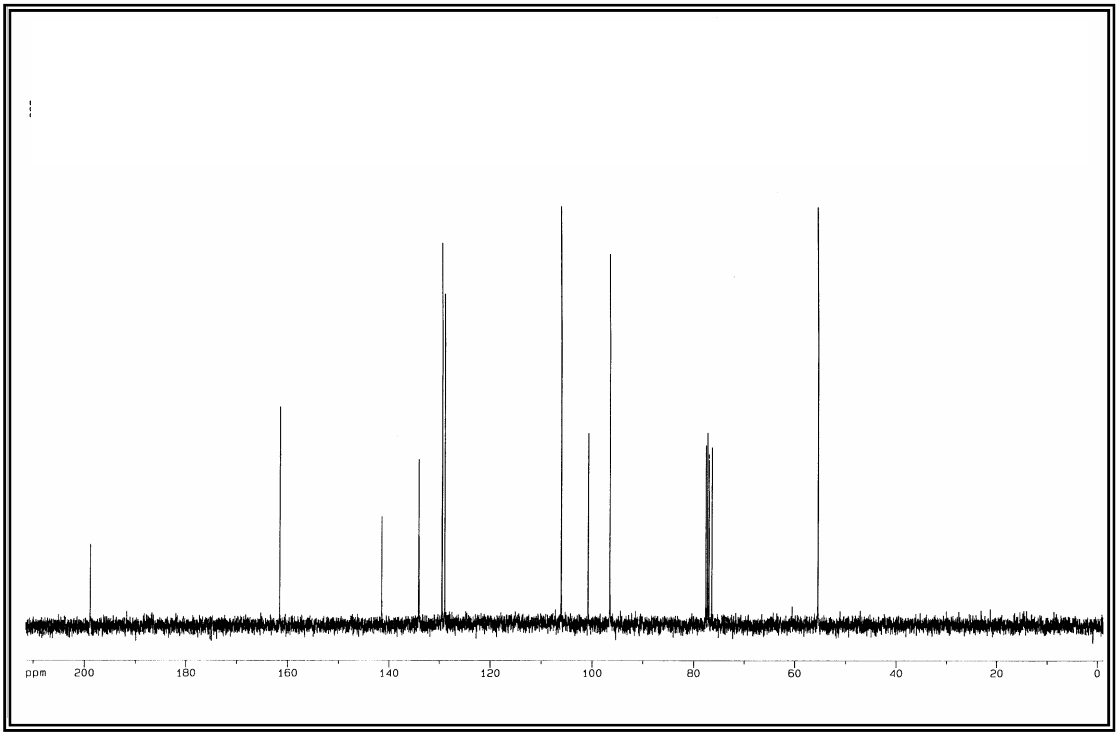


Figure 45: ^{1}H and ^{13}C NMR spectra of (53a)

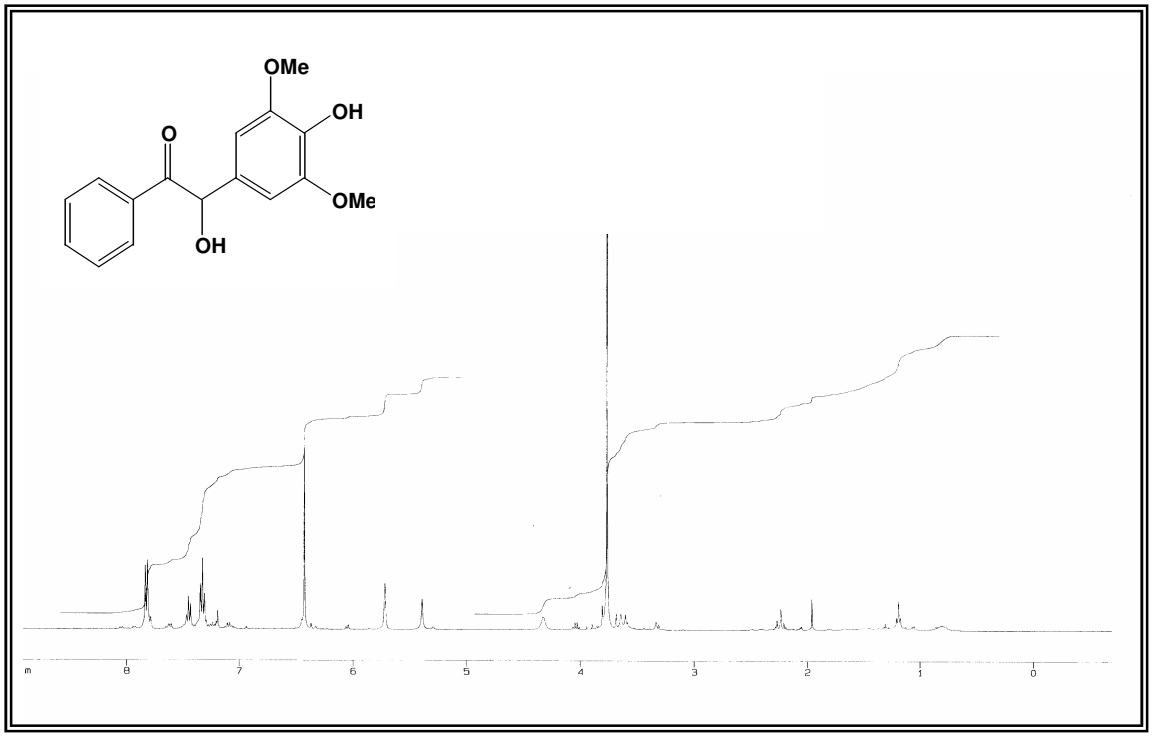


Figure 46: ^{1}H NMR spectrum of (53b)

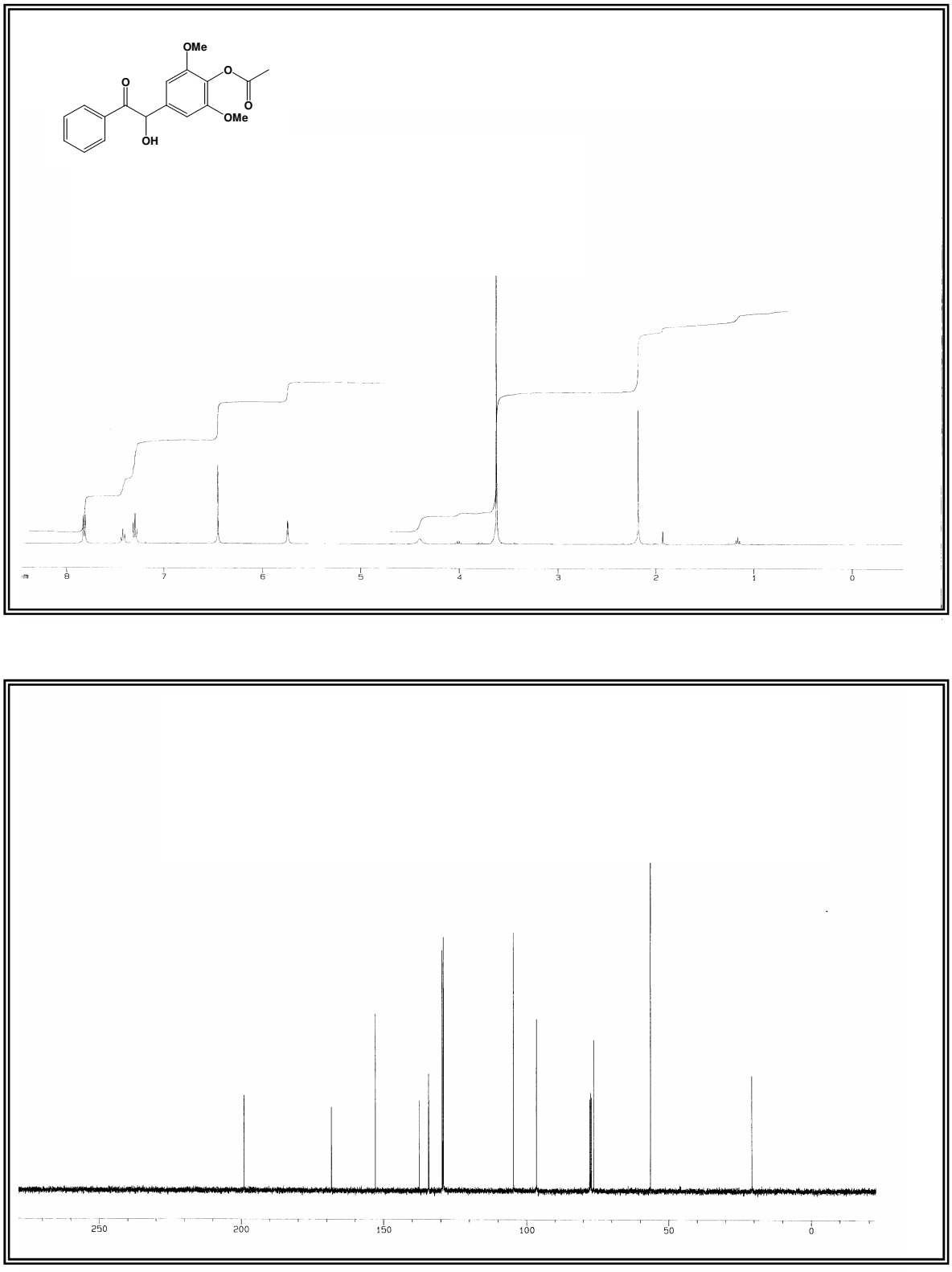


Figure 47: ^{1}H and ^{13}C NMR spectra of (53c)

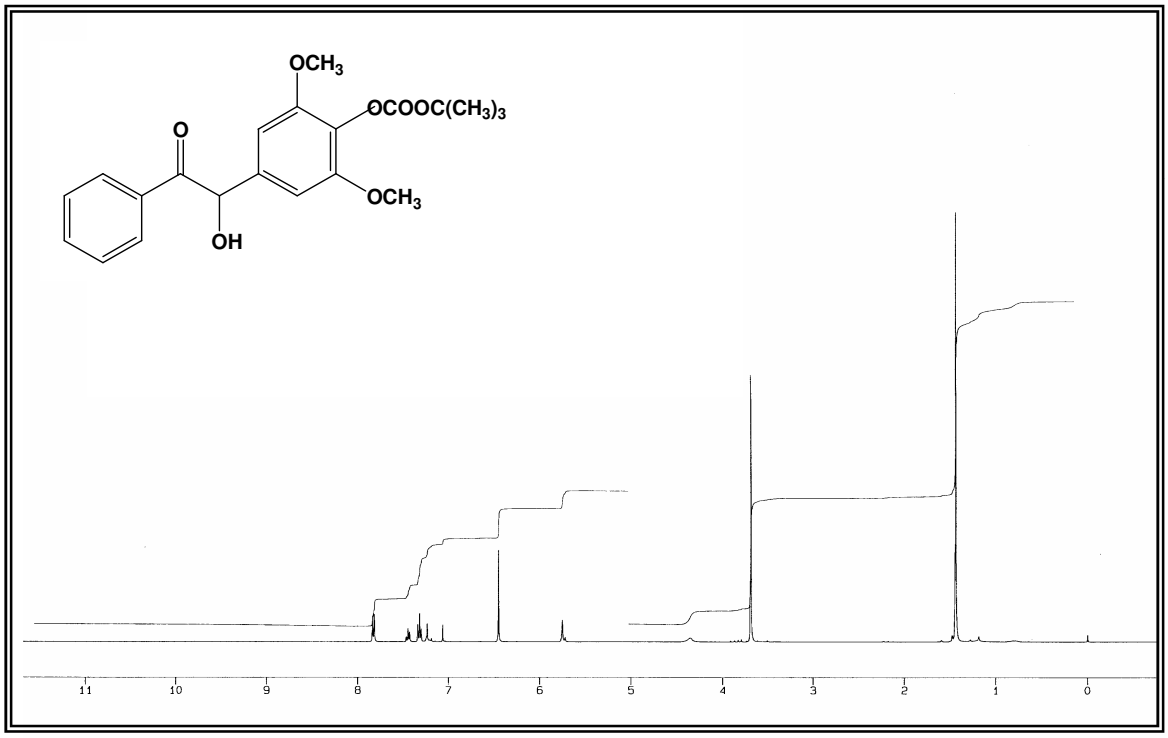


Figure 48: ^{1}H NMR spectrum of (53d)

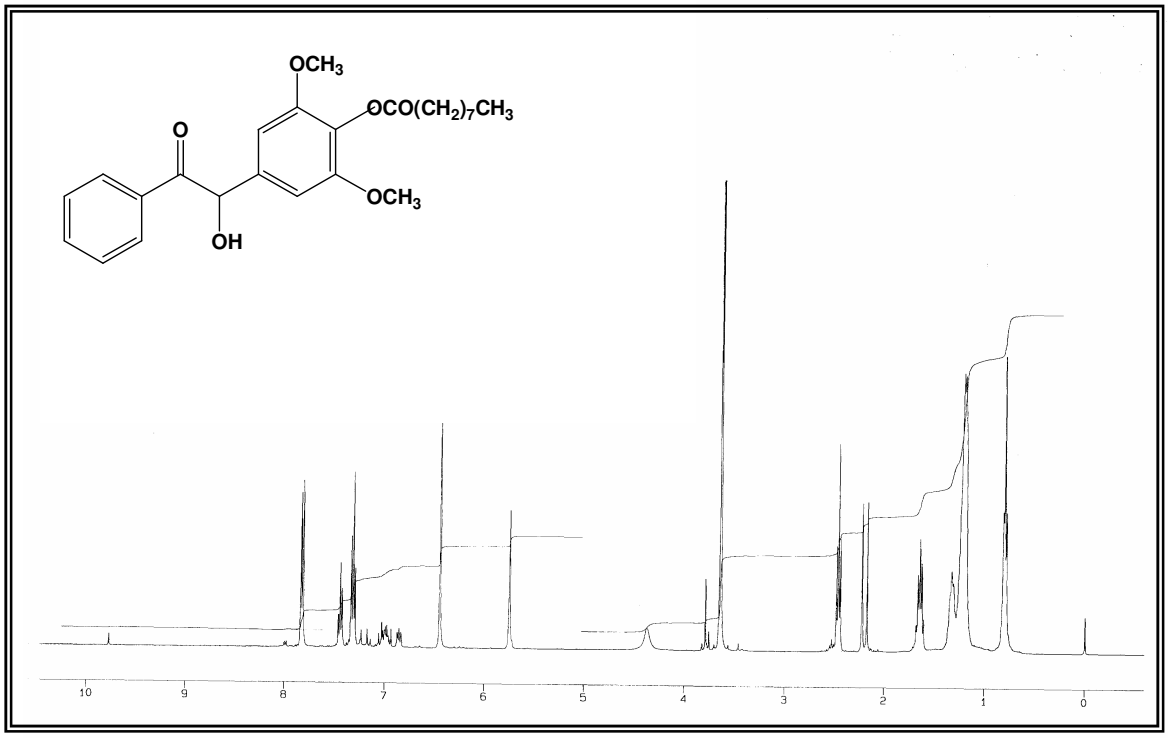


Figure 49: ^{1}H NMR spectrum of (53e)

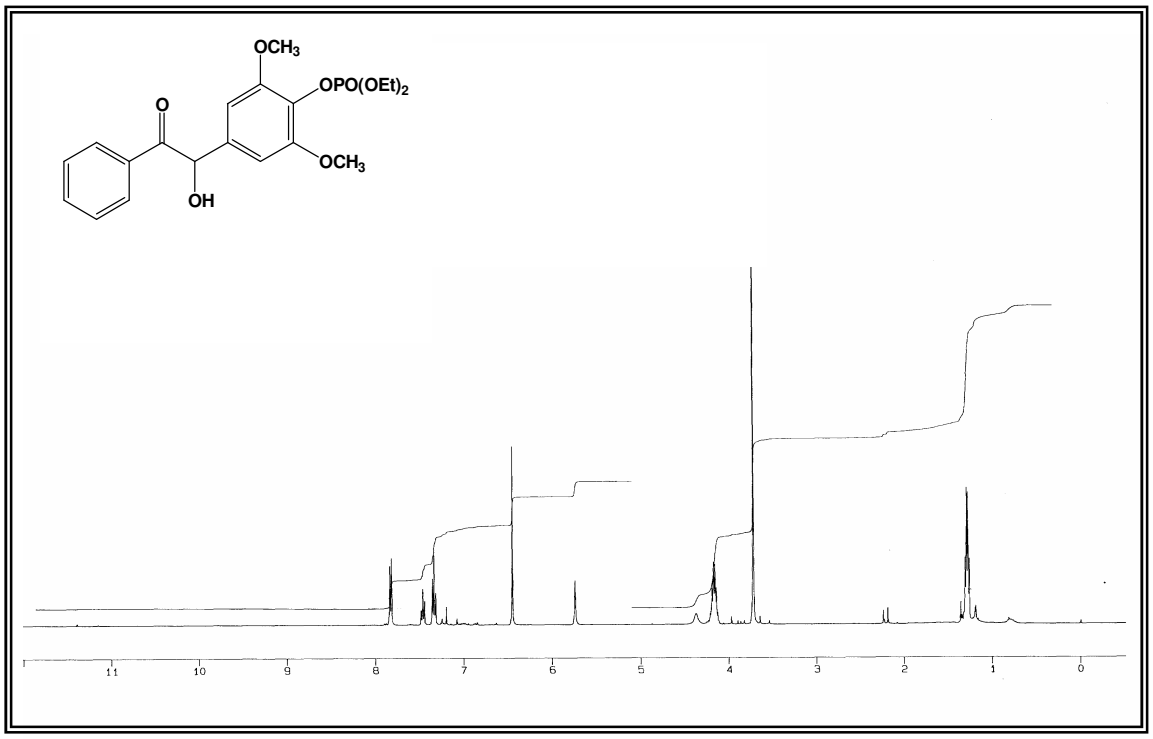
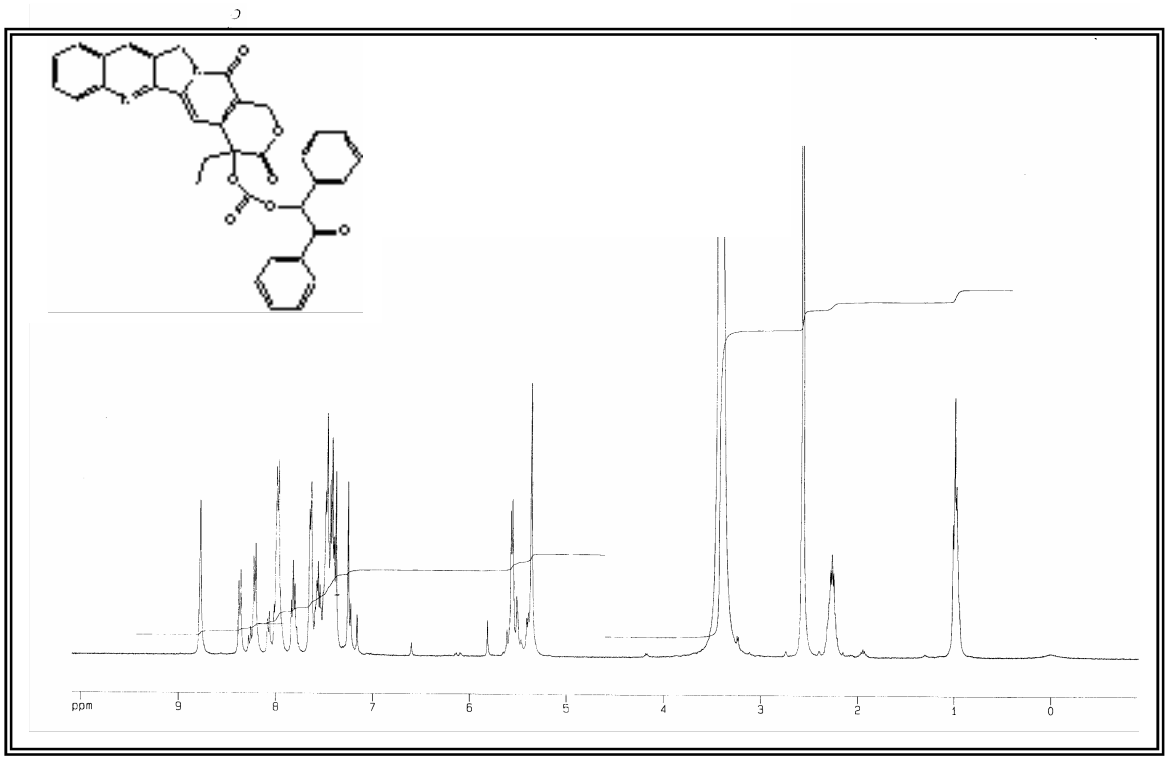


Figure 50: ^{1}H NMR spectrum of (53f)



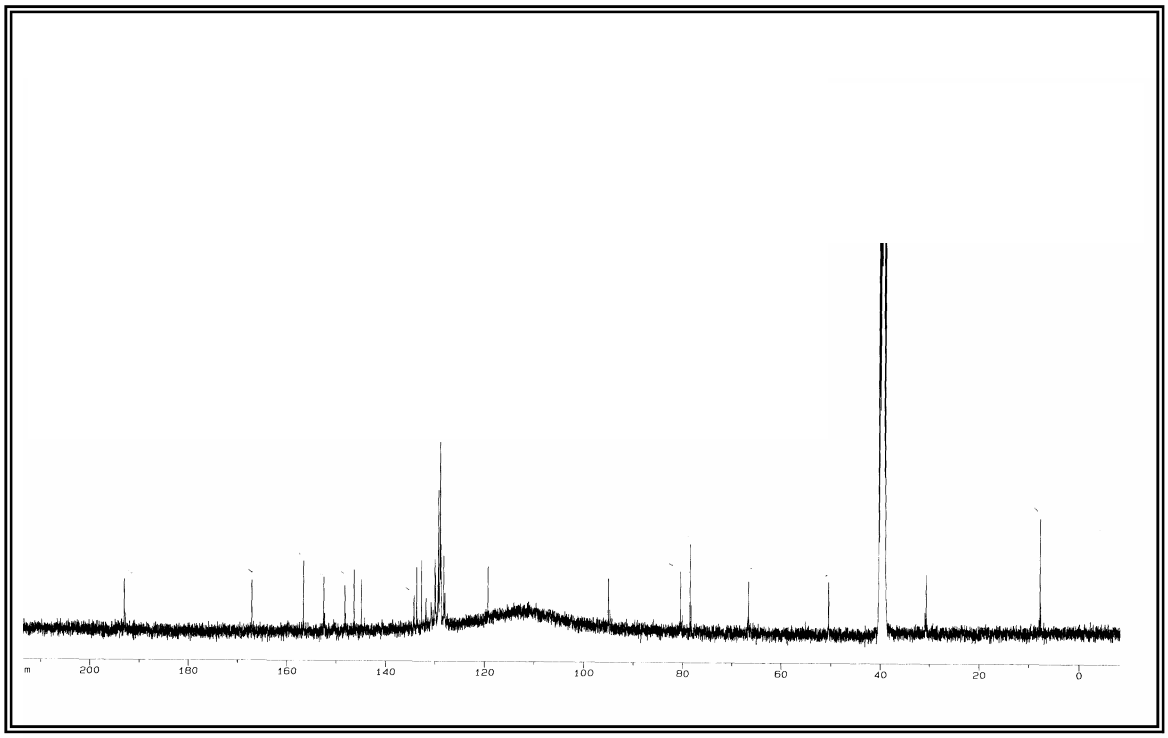
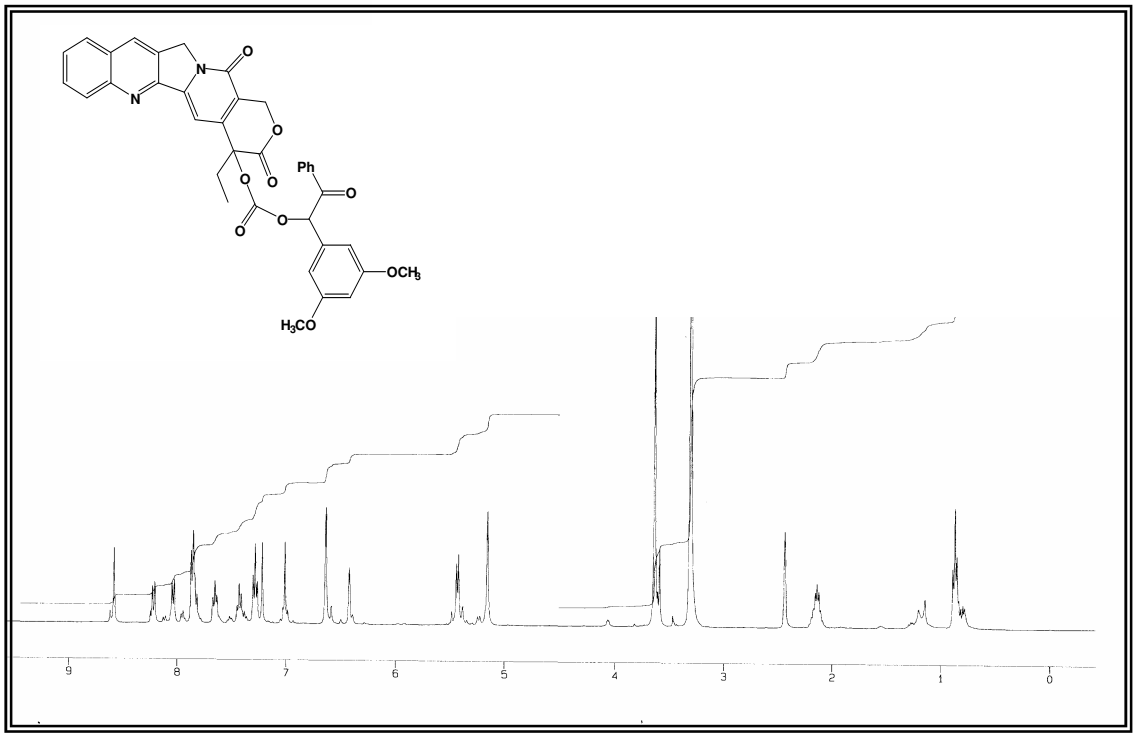


Figure 51: ^{1}H and ^{13}C NMR spectra of (55)



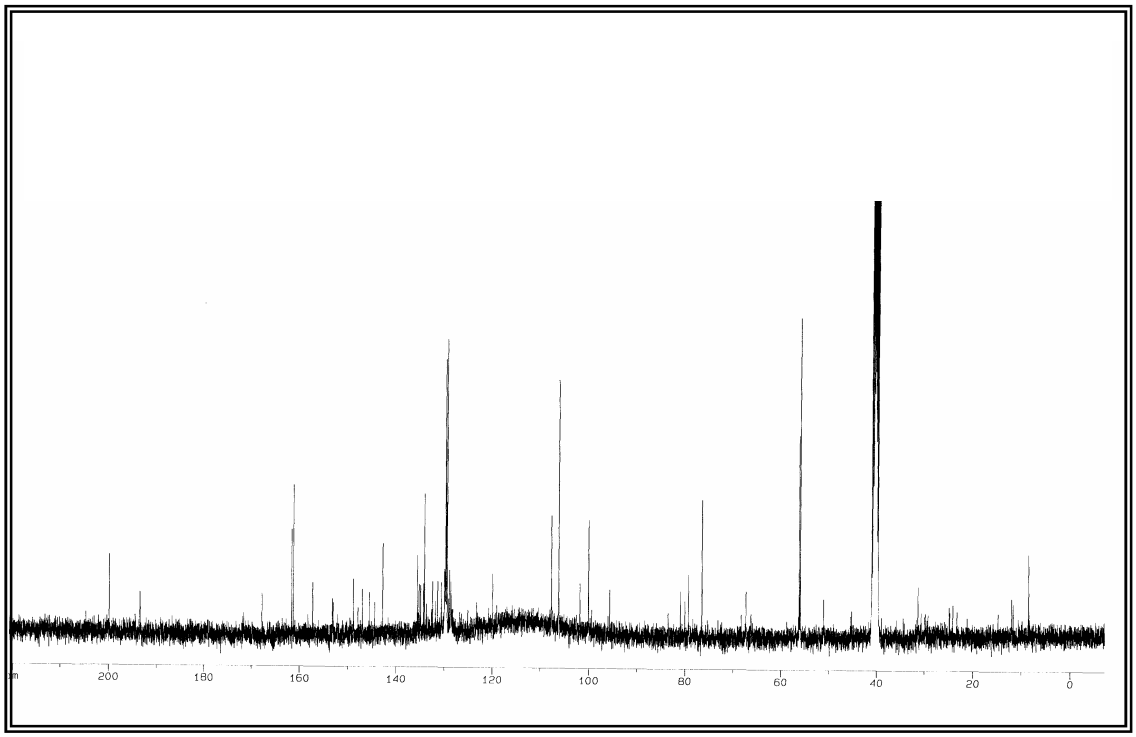


Figure 52: ^{1}H and ^{13}C NMR spectra of (57a)

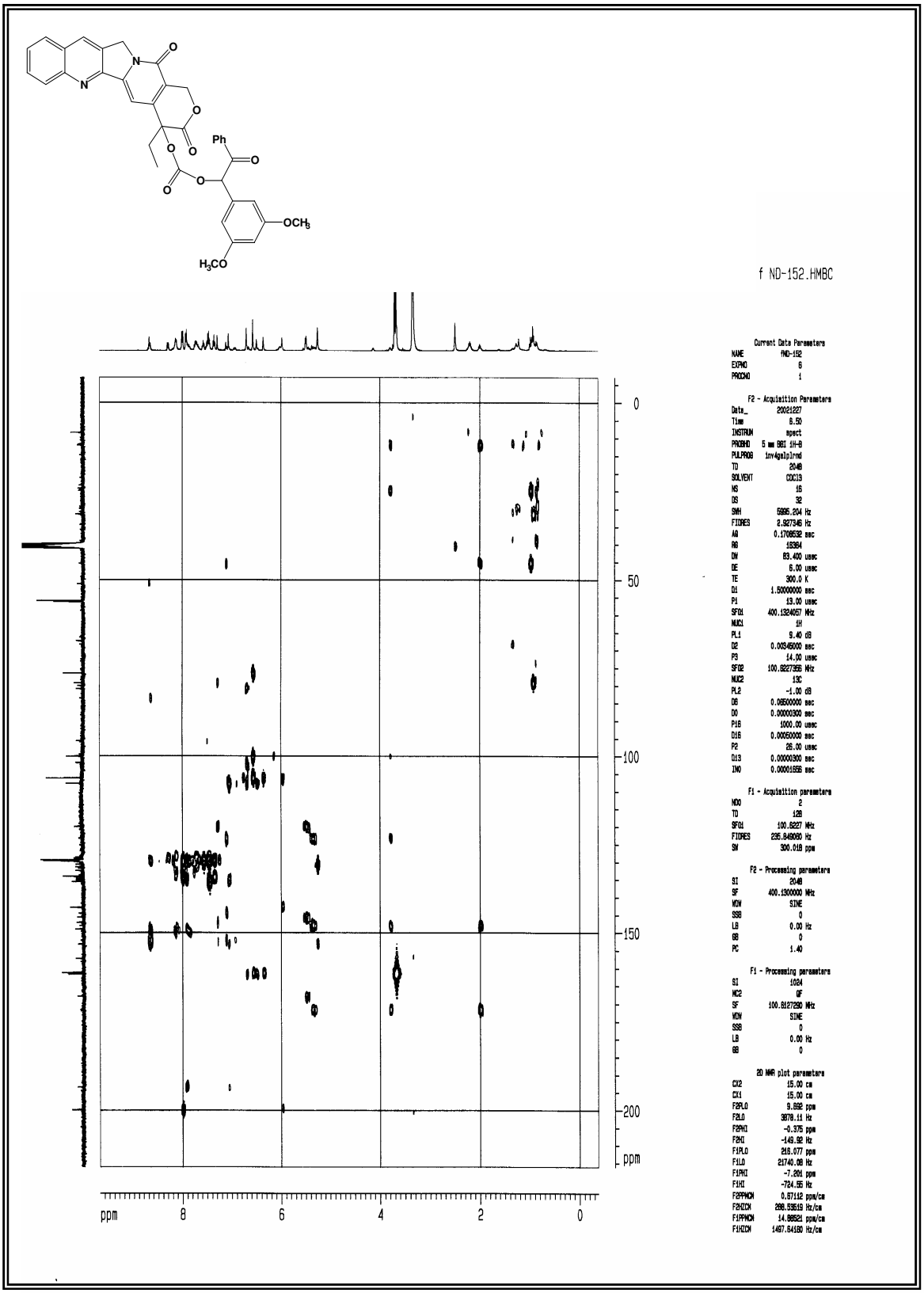


Figure 53: HMBC spectrum of (57a)

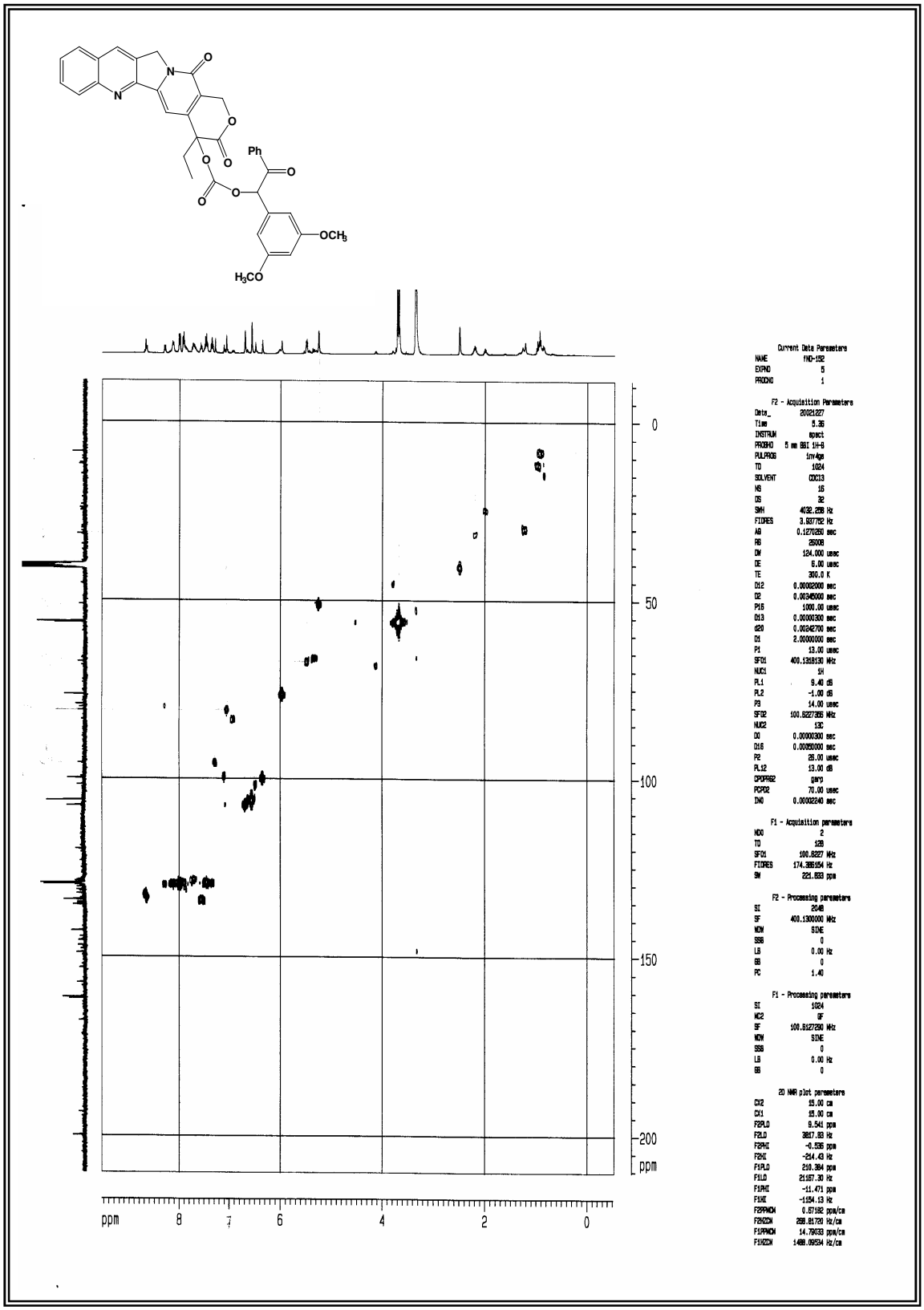


Figure 54: HMQC spectrum of (57a)

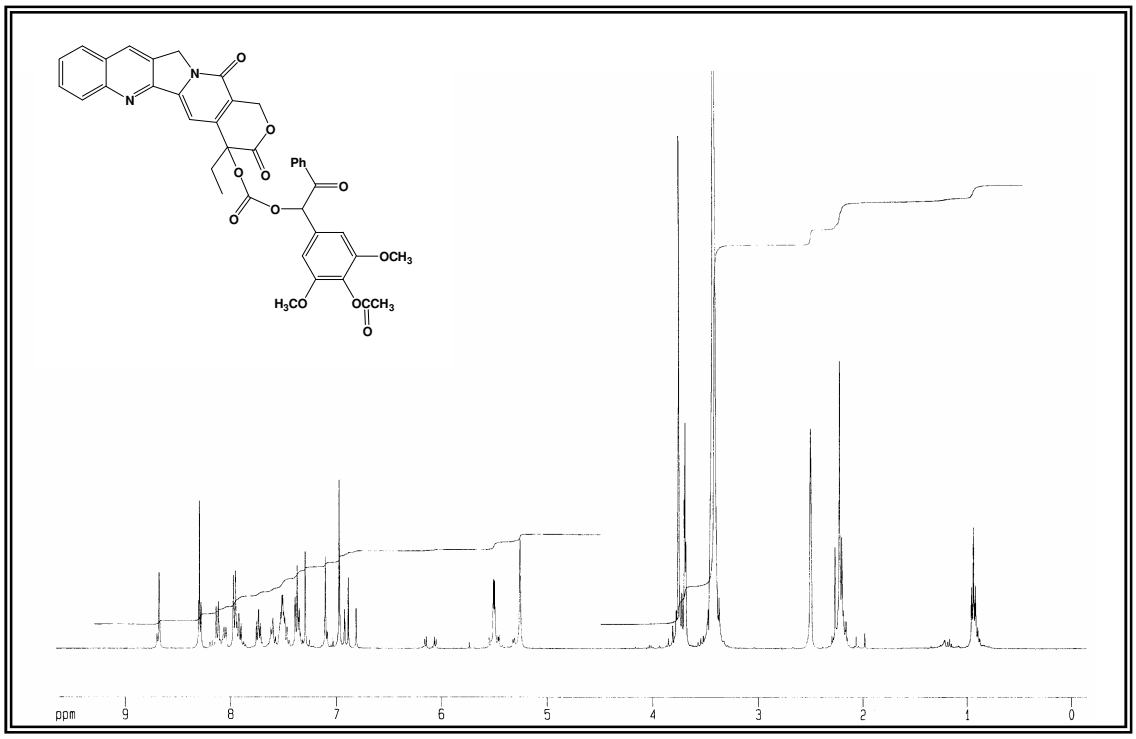


Figure 55: ^{1}H NMR spectrum of (57b)

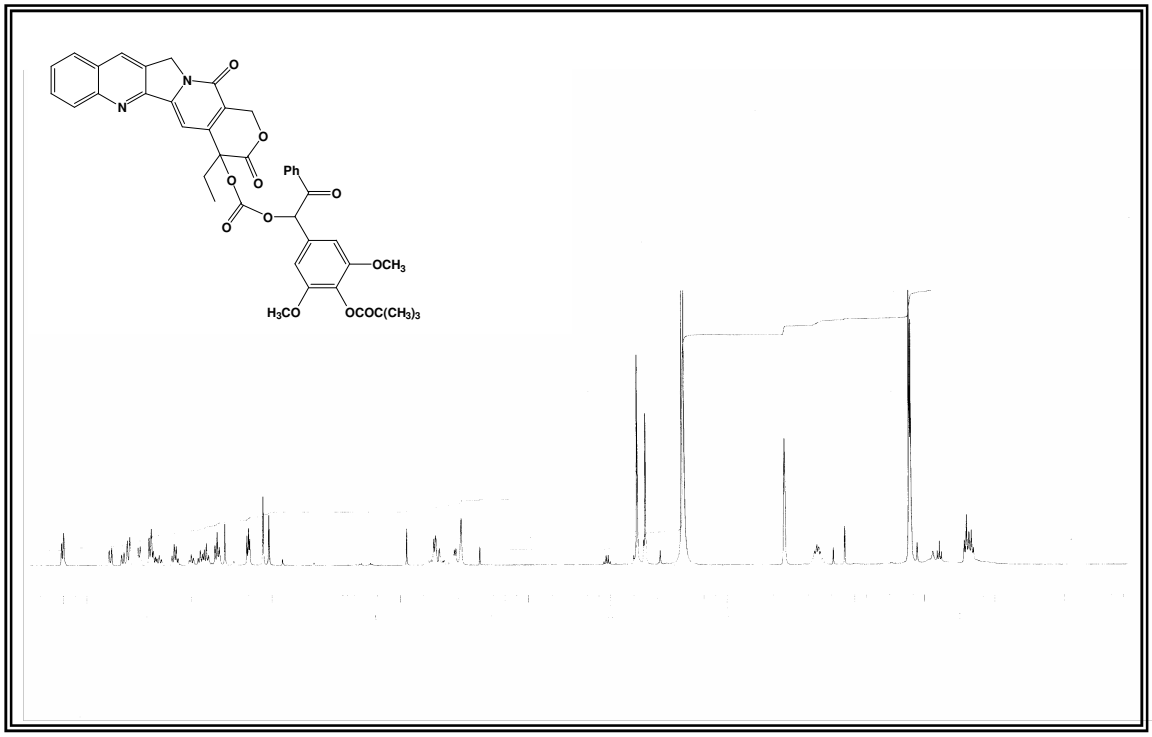


Figure 56: ^{1}H NMR spectrum of (57c)

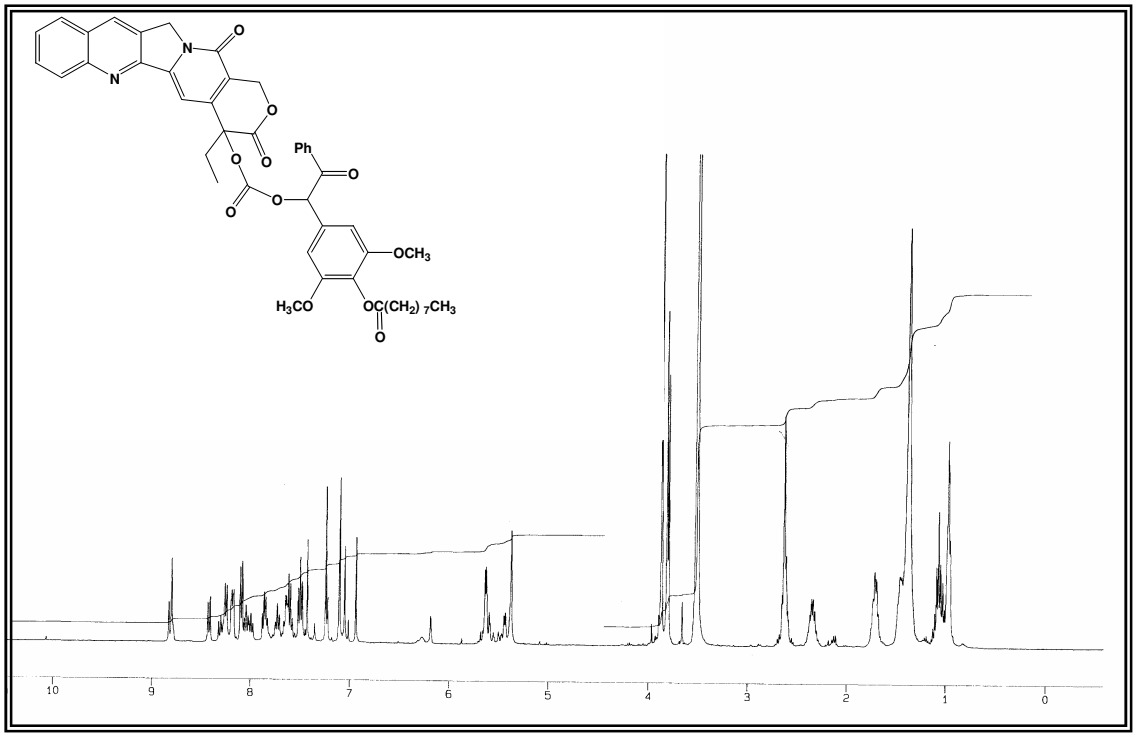


Figure 57: ^{1}H NMR spectrum of (57d)

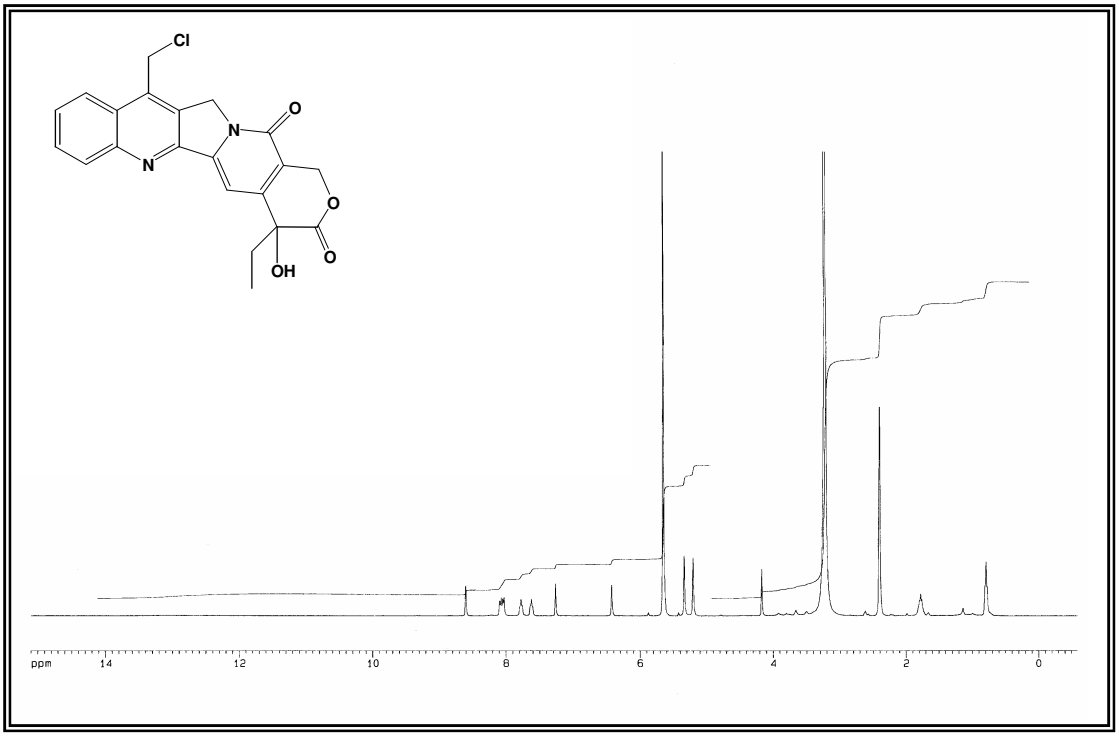


Figure 58: ^{1}H NMR spectrum of (58)

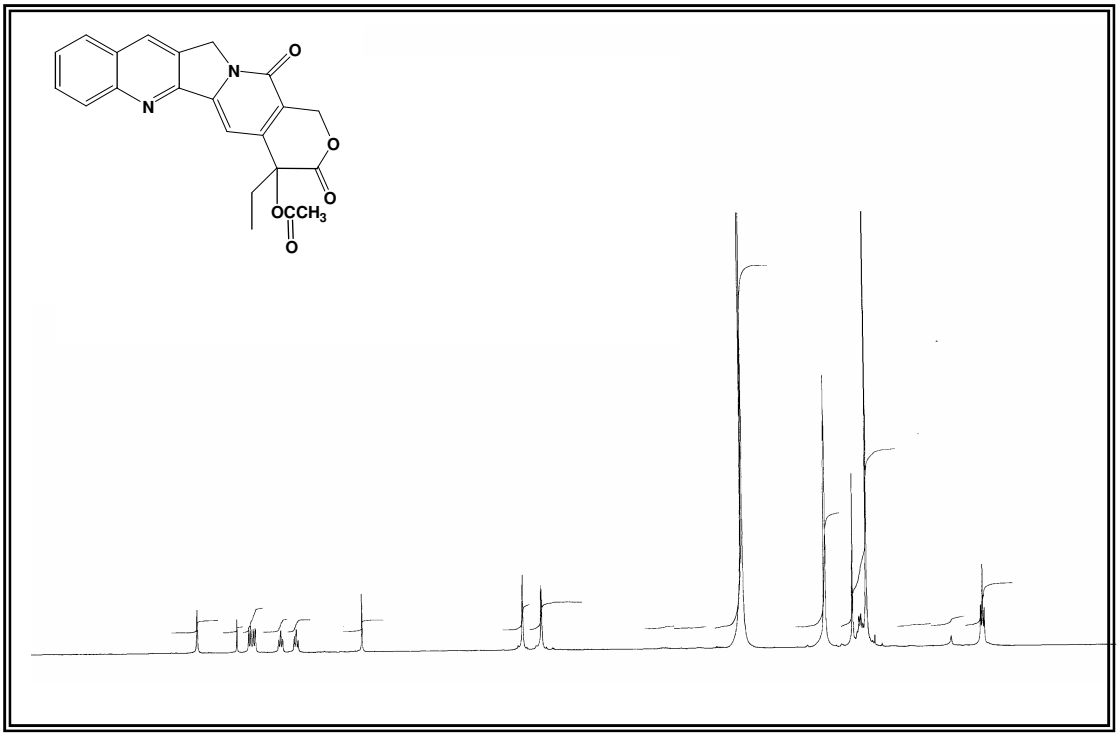


Figure 59: ^{1}H NMR spectrum of (60)

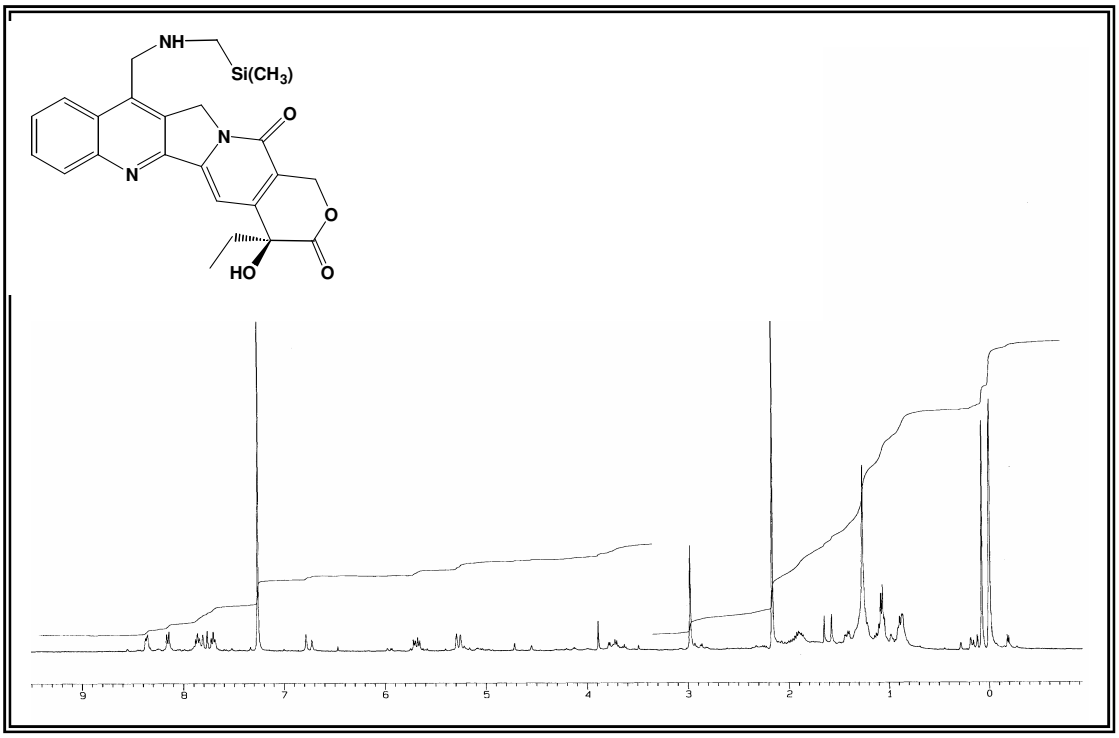


Figure 60: ^{1}H NMR spectrum of (61)

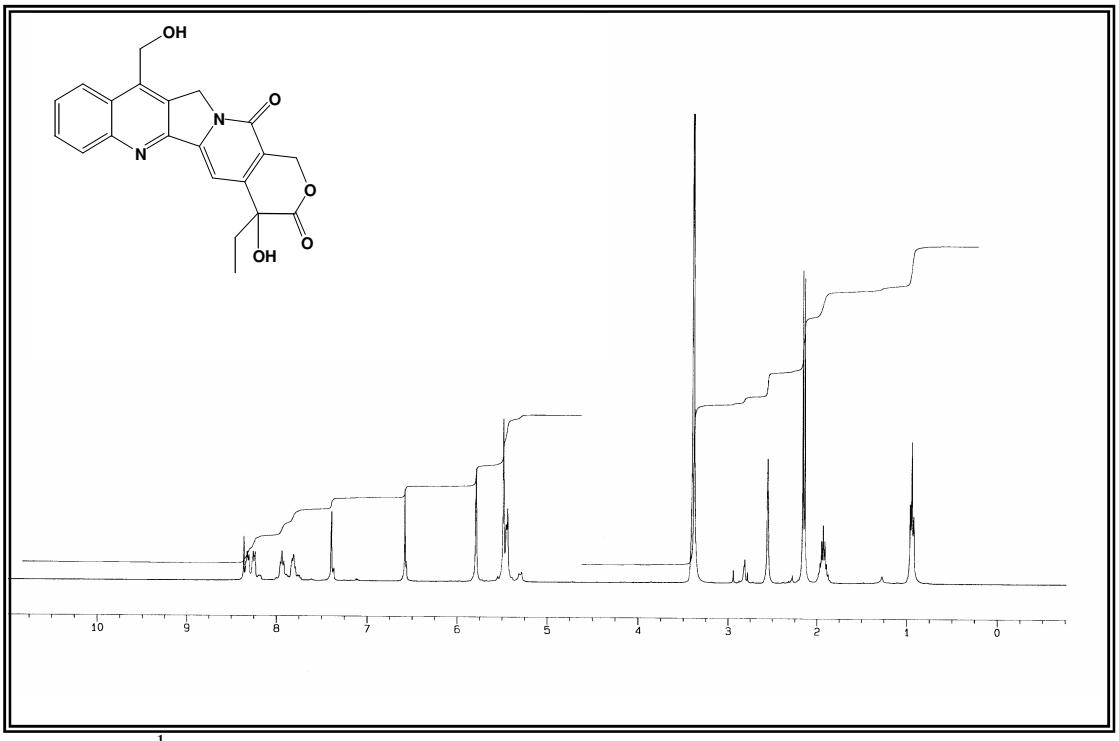


Figure 61: ^{1}H NMR spectrum of (62)

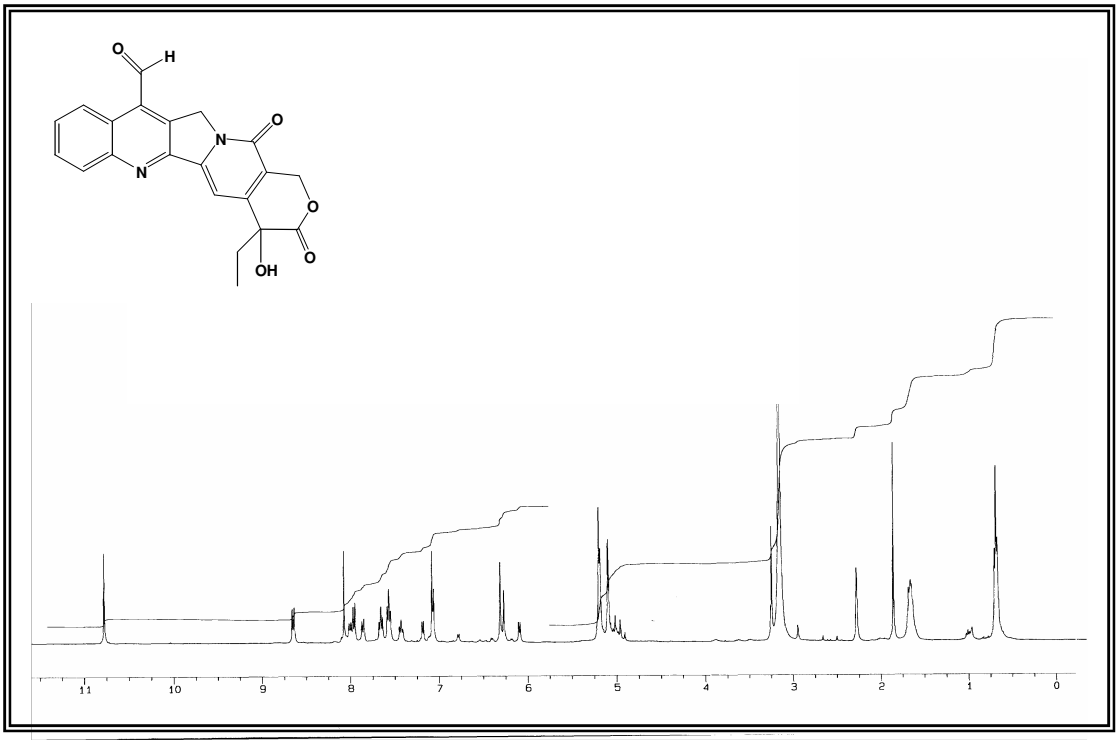


Figure 62: ^{1}H NMR spectrum of (63)

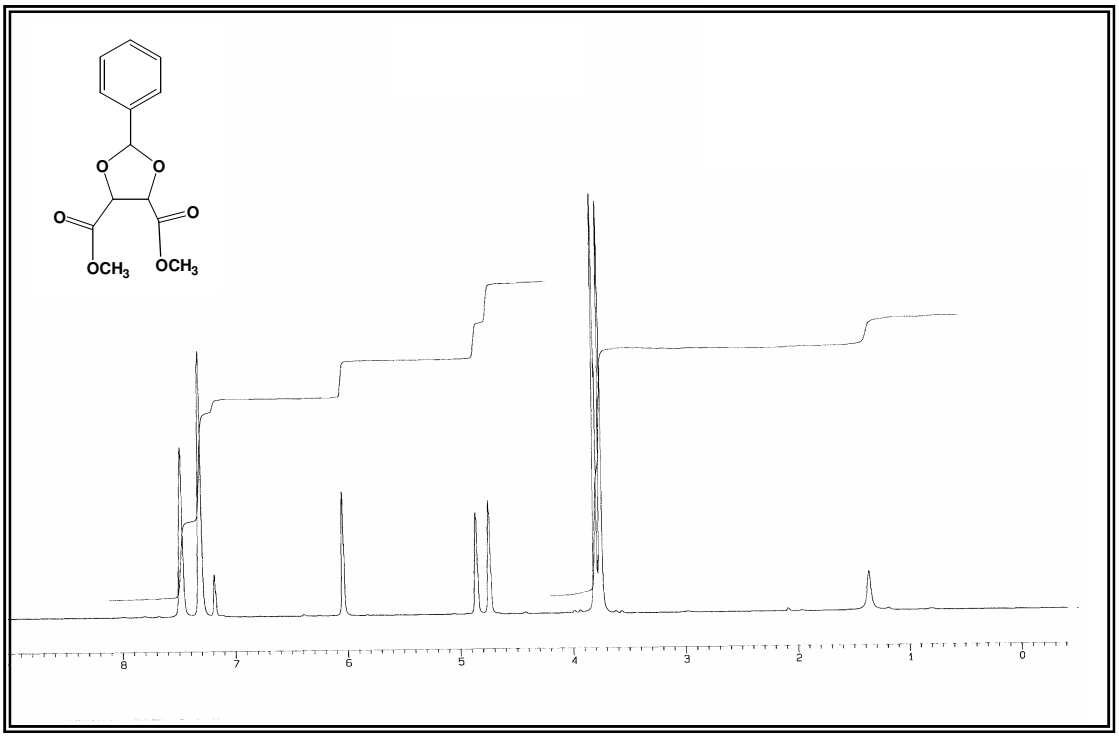


Figure 63: ^{1}H NMR spectrum of (71)

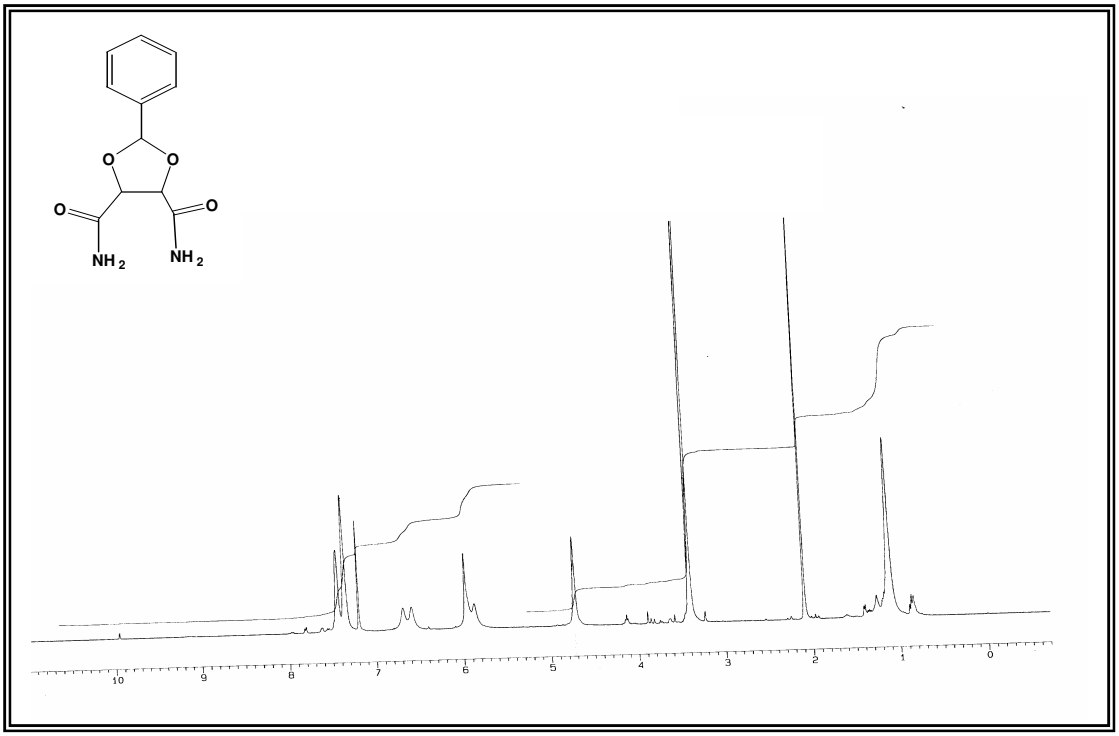


Figure 64: ^{1}H NMR spectrum of (72)

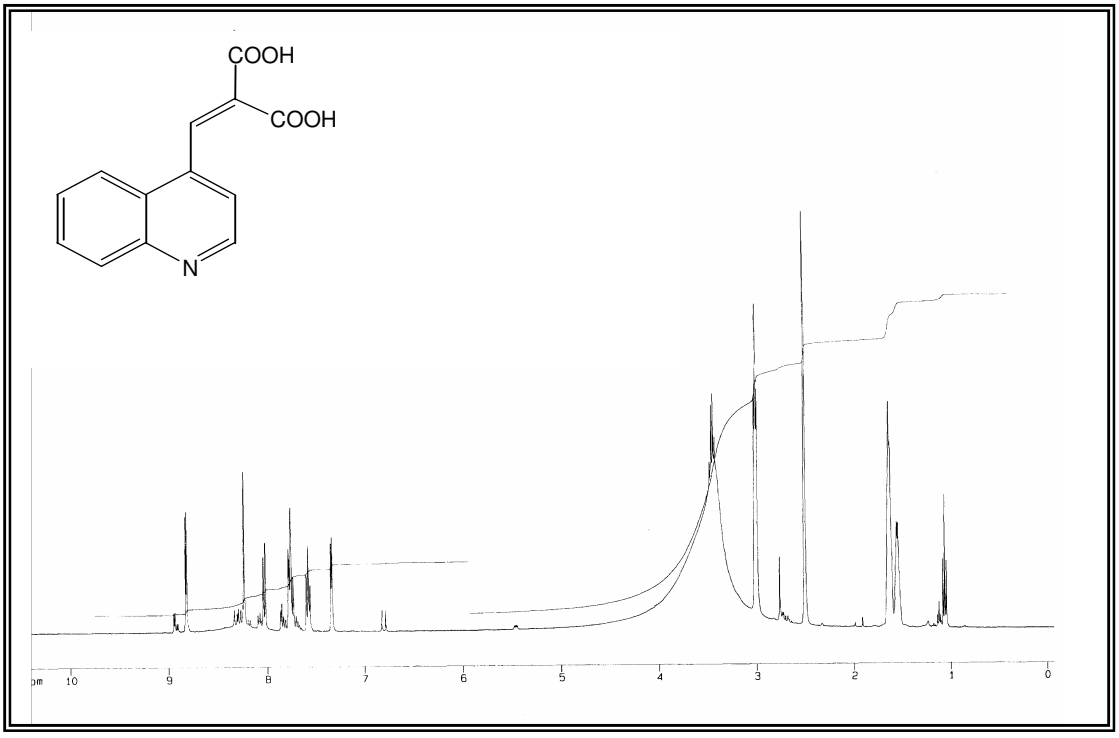


Figure 65: ^{1}H NMR spectrum of (74)

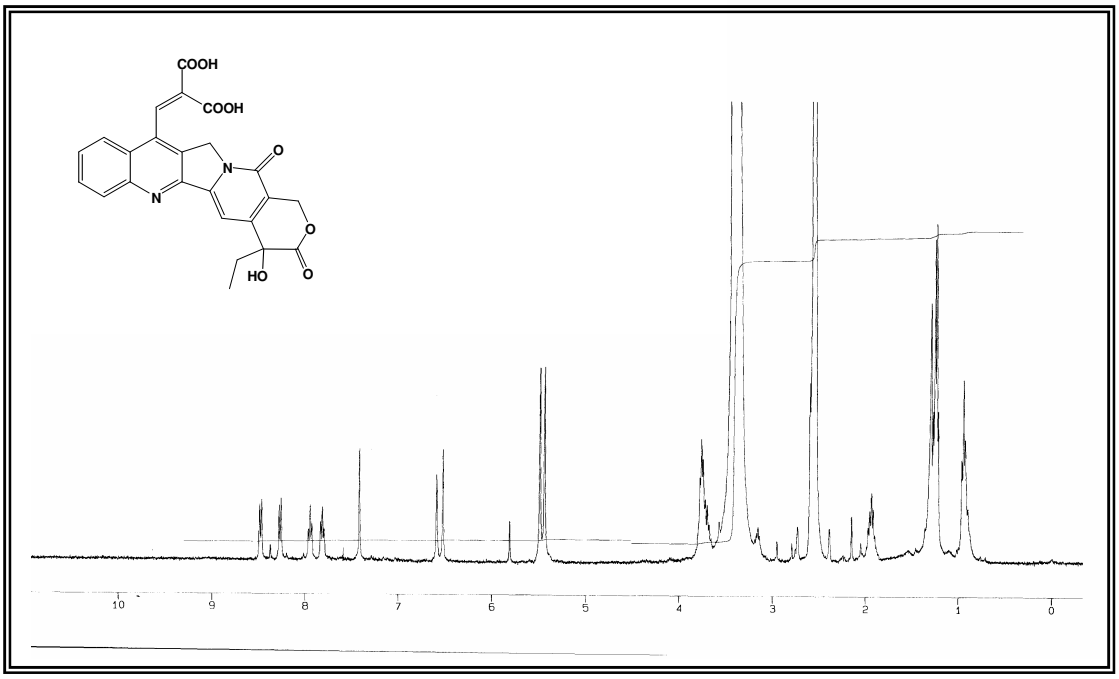


Figure 66: ^{1}H NMR spectrum of (77)