ASYMMETRIC SYNTHESIS AND ANTI-TUMOR PROPERTIES OF CONFORMATIONALLY CONSTRAINED ANALOGUES OF (S)- AND (R)-GONIOTHALAMIN

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ABSTRACT

ASYMMETRIC SYNTHESIS AND ANTI-TUMOR PROPERTIES OF CONFORMATIONALLY CONSTRAINED ANALOGUES OF (S)- AND (R)-GONIOTHALAMIN

Naturally isolated 5-substituted-α,β-unsaturated-δ-lactones gained great attention of researchers due to their cytotoxic and anti-tumor properties. Styryl lactones are the most interesting members of this group of naturally available compounds. One of the well-known and important example for styryl lactone is goniothalamin, which shows cytotoxicity against variety of cancer cell lines. This cytotoxic property was shown to be selective for cancer cell lines with no significant cytotoxicity toward non-malignant cells. Recent structure activity relationship (SAR) studies on goniothalamin shows that R configuration on its stereogenic center, trans double bonded linker and Michael acceptor parts of the molecules are essential for its cytotoxic activity.

In this study conformationally constrained analogues of (S)- and (R)-goniothalamin were synthesized. Syntheses were started with the catalytic asymmetric allylation of benzaldehyde, naphthaldehyde and quinaldehyde derivatives in the first step, then formed alcohols were acrylated with acryloyl chloride to yield the corresponding esters, in the last step, ring closing metathesis with Grubbs' catalyst yielded the target molecules. Meanwhile, in this study the synthesized 5-aryl-substituted-α,β-unsaturated-δ-lactones were tested to determine their cytotoxicity against MCF-7, PC-3, DU-145 and LNCAP cancer cell lines.

ÖZET

KONFORMASYONEL OLARAK SABİTLENMİŞ (S)- VE (R)-GONİOTHALAMİN TÜREVLERİNİN ASİMETRİK SENTEZLERİ VE ANTİ-TÜMÖR ÖZELLİKLERİNİN İNCELENMESİ

Doğal yollardan izole edilen 5-sübstitüentli-α,β-doymamış-δ-laktonlar sahip oldukları sitotoksik ve anti-tümör etkisi sayesinde günümüzde araştırmacıların büyük ilgisini kazanmıştır, bunlar arasından en ilginci stiril laktonlardır. Stiril lakton ailesinin önemli üyelerinden biri olan goniothalaminin değişik kanser hücrelerine karşı yüksek sitotoksiteye sahip olduğu gözlemlenmiştir. Bunun yanında goniothalamin sağlıklı hücreler üzerinde de test edilmiş ve minimal etki gözlemlenmiştir. Bu nedenle goniothalaminin kanser hucrelerine karşı seçici sitotoksisite gösterdiği düşünülmektedir. Yakın zamanda goniothalamin üzerinde gerçekleştirilmiş olan yapı-aktivite ilişkileri çalışmalarından yapısındaki R konformasyonuna sahip asimetrik merkezin, Michael akseptoru ve sübstitüent kısmındaki trans-çifte bağın sitotoksik etkinin korunması için gerekli olduğu gözlemlenmiştir.

Bu çalışmada (S)- ve (R)-goniothalamin'in stiril kısmı, konformasyonel değişimleri engelleyecek şekilde yeni aromatik sübstratlarla yer değiştirilmiştir. Sentezler ilk basamakta benzaldehit, naftaldehit ve kinolaldehit türevlerinin katalitik asimetrik alillenmesi ile başlamaktadır, daha sonra oluşan alkol türevleri akrilklorür yardımı ile esterleştirilmiştir ve son basamakta Grubbs' katalizörü eşliğinde halka kapanması tepkimesiyle hedef moleküllerin sentezleri gerçekleştirilmiştir. Aynı zamanda bu çalışmada sentezlenen 5-aril sübstütientli-α,β-doymamış-δ-laktonların MCF-7, PC-3, DU-145 and LNCAP gibi farklı kanser hücreleri üzerindeki sitotoksik etkileri test edilmiştir.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLE

CHAPTER 1

INTRODUCTION

Cancer is the general name for a group of more than 100 diseases in which cells in a part of the body begin to grow out of control. Normal body cells grow, divide, and die in an orderly fashion and during the early years of a person's life, normal cells divide more quickly until the person becomes an adult. After that, cells in most parts of the body divide only to replace worn-out or dying cells and to repair injuries.

Most of the time when DNA becomes damaged, either the cell dies or is able to repair the DNA. In cancer cells, the damaged DNA is not repaired and these cells continue multiplying although the body doesn't need them, the result is called as tumor. These tumors are considered either benign or malignant, benign is considered non-cancerous and malignant is cancerous (Gringauz 1996)

Cancers can begin in many different parts of the body and have the ability to spread other organs where it is starting from. This process is called as metathesis. Each cancer type can act very differently depending on the place where it grows, spread or grow at different rates and respond to different treatments. The treatment choices are also depend on the type of cancer, the stage of the cancer, and other individual factors.

Mainly, a person's DNA gets damaged by things in the environment, like, chemicals, viruses, tobacco smoke or too much sunlight. These risk factors are increasing the probability of facing with theat of cancer in human life.

In the year 2000, World Health Organization announced that, malignant tumours were responsible for 12 per cent of the nearly 56 million deaths worldwide from all causes and in many countries, more than a quarter of deaths are attributable to cancer. When it is expressed with numbers in 2000, 5.3 million men and 4.7 million women developed a malignant tumour and died from the disease (World Health Organization Report 2003).

It is envisioned that, countries which have middle or low income will face with the deaths resulting from cancer over the next 25 years, and it will increase from 7.4 to 11.8 million by World Health Organisation Comminity according to their World Health Statistics 2008 report.

As cancer is being one of the most severe health problems worldwide, the studies on development of new anticancer drugs and different treatment strategies gain importance in the field of science. For the development of new anticancer drugs, natural products play a dominant role with more than 60% of anticancer compounds being either natural products or derived from natural products (Newman, et al. 2003). Today there are many effective anticancer agents in current use which have provided by nature.

The World Health Organization estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs. (Farnsworth, et al. 1985)

Today, drugs of natural origin have been classified as original natural products, products derived semisynthetically from natural products, or synthetic products based on natural product models. (Cragg, et al. 1997)

Many clinically useful drugs have been discovered from various plants and they were an excellent source for many diseases. Researches on this area shows that plant are also good source for anticancer agents. Natural products or their semi-synthetic analogues constitute approximately 74% of all new chemical entities marketed as anti-cancer drugs between 1981 and 2006 (Newman and Cragg 2007)

The genus *Goniothalamus (Annonaceae)* is one of the this inexhaustable source of natural compounds, consists of 115 species and these plants has been used for timber, as fiber sources and a mosquito repellant and most interestingly in folk medecine. (Surived and Vatéle 1999)

This species are classifed in three groups, including styryl lactones, annonaceous acetogenin and alkaloids. Alkaloids and annonaceous acetogenin anti-tumor activity was detected previously and some of these species were widely used as traditional medicines. (Tian, et al. 2006)

The another class of compound extracted and isolated from the Goniothalamus is styryl lactones which gained the attention of researchers in last decade due to the strong cytotoxicity to different cancer cell lines.

When we think about the kinds of plant species on earth, the number of the plants have been exhaustively studied for their potential value as a source of drug is too limited. These show us that nature is still extremely important source for natural drugs. However the challenges of drug discovery, formidable effort, time, and expense are

required for the complex development processes that move a new agent from discovery to its approval for use in the treatment. Nevertheless, this endeavour is necessary for the mankind.

In this study we aimed to synthesize novel (R) and (S)-substituted α,β-unsaturated δ-lactone derivatives enantioselectively and investigate their anti-tumor activities.

CHAPTER 2

BIOLOGICAL PROPERTIES OF STYRYL LACTONES

Nature is an inexhaustible source of natural compounds with interesting biological activities, because of these crucial properties the researches on drug discovery mainly focused on the isolation and analysis of compounds from nature, mostly from plant origins. Actually, the extracts or the isolated compounds from nature serve as the primary source for the medicine for centuries.

Due to the progress in science, synthesis of the natural plant products could be performed in the last century. However, still many of the biologically active plant compounds fully gained from on plant resources due to their complexity. This complex structures of compounds directed the chemists to search for an important source of new compounds with a variety of structural arrangements and singular properties.

Many natural products with different biological activities, have α,β-unsaturated δ-lactone moieties as an important structural feature. The α,β-unsaturated δ-lactone functionality is presumed to be responsible for the biological activities, due to its ability to act as a Michael acceptor, enabling these molecules to bind to a target enzyme. (Enders and Steinbusch 2003)

Styryl lactones, which have an α,β-unsaturated-δ-lactone moieties in their structure, are an interesting group of cytotoxic and antitumor agents. Because of their unique and intriguing structures and the broad spectrum of activity, they have attracted the attention of several researchers.

In decades, many biologically active styryl lactones that have been isolated or synthesized from *Goniothalamus*, exhibit promising anti-tumor activity. (Bermejo, et al. 1999.) A few bioactivity studies were done with the compounds goniothalamin (**1**), goniodiol (**2**), and altholactone (**3**) isolated from *Goniothalamus griffithii* and all three styryl lactones have been found to possess cytotoxic activities. Among the styryl lactones altholactone (**3**), and goniopypyrone (**4**) are the most cytotoxic styryl lactones, which have IC_{50} values 86.2 μM for HL-60 cells and non selective cytotoxicity of

goniopypyrone (**4**) with ED_{50} values 0.67 µg/ml for several human tumor cell line (Figure 2.1) (Inayat-Hussain, et al. 2002, Surivet and Vatele 1997, Tian, et al. 2006).

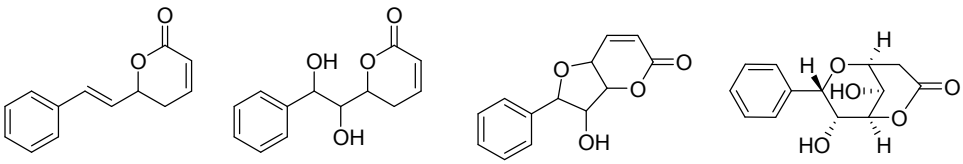


Figure 2.1. Structures of goniothalamin (**1**), goniodiol (**2**), altholactone (**3**), and goniopyrpyrone (**4**).

Obolactone (**5**) and obochalcolactone (**6**) have recently been isolated from the fruits and the trunk bark of *Cryptocarya obovata*, they also display significant cytotoxic activity against the KB cell line with an IC_{50} values of 3 and 5 μM respectively (Figure 2.2) (Dumontet, et al. 2004).

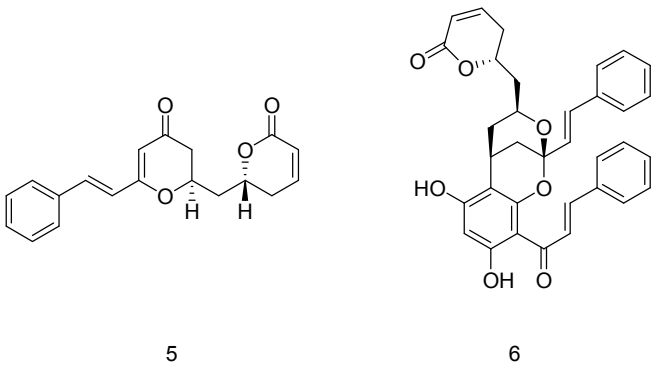


Figure 2.2. Structures of obolactone (**5**) and obochalcolactone (**6**).

The styryl lactones can be classified in six groups based on the structural characteristics of the skeletons. These groups are; styryl-pyrones, furano-pyrones, furano-furones, pyrano-pyrones, butenolides, and heptolides (Bermejo, et al. 1999).

2.1. Furano-pyrones

The first identified compound of this group was altholactone (**3**). It was also isolated from the bark of *Goniothalamus giganteus* and reported under the different trivial name of goniothalenol (El-Zayat, et al. 1985). Altholactone (**3**) and all furanopyrones are biogenetically related to styryl-pyrones. The furano-pyrone skeleton represents the second most abundant class of styryl-lactones in *Goniothalamus*. Isoaltholactone (**7**), 2-epi-altholactone (**8**), goniofupyrone (**9**), goniotharvensin (**10**), and etharvensin (**11**) are the other members of this group (Figure 2.3) (Bermejo, et al. 1999).

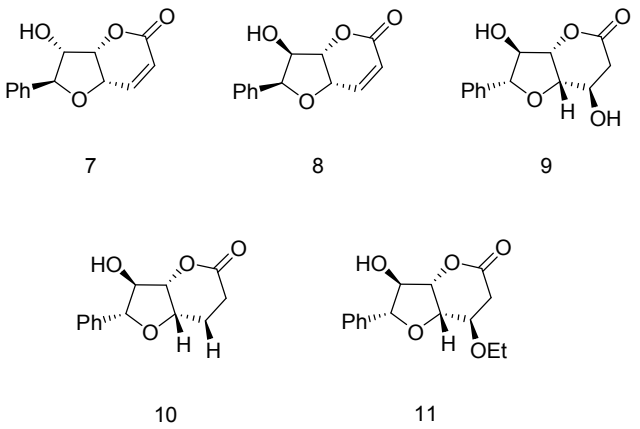
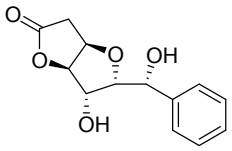


Figure 2.3. Structures of isoaltholactone (**7**), 2-epi-altholactone (**8**), goniofupyrone (**9**), goniotharvensin (**10**), and etharvensin (**11**).

2.2. Furano-furones

Goniofufurone (**12**), and 7-epi-goniofufurone (**13**) are members of this group of styryl lactone (Figure 2.4). They were isolated from the stem bark of *Goniothalamus giganteus* (Bermejo, et al. 1999). Fang and coworkers reported the cytotoxic activity of compound (**12**) against A-549 (human lung carcinoma), MCF-7 (human breast adenocarcinoma), and HT-29 (human colon adenocarcinoma) cell lines with $ED_{50} < 4$ μg/ml (Fang, et al. 1991).



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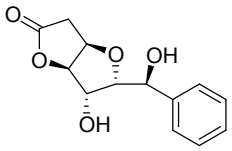

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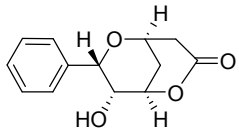
Figure 2.4. Structures of goniofufurone (**12**), and 7-epi-goniofufurone (**13**).

2.3. Pyrano-pyrones

The examples of pyrano-pyrone styryl-lactones are goniopypyrone (**4**), 5-deoxy-goniopypyrone (**14**), and leiocarpin-A (**15**). They are also exhibiting non-selective activity against human tumour cell lines (Figure 2.5) (Bermejo, et al. 1999).



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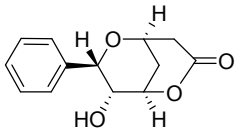

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Figure 2.5. Structures of 5-deoxy-goniopypyrone (**14**), and leiocarpin-A (**15**).

2.4. Butenolides

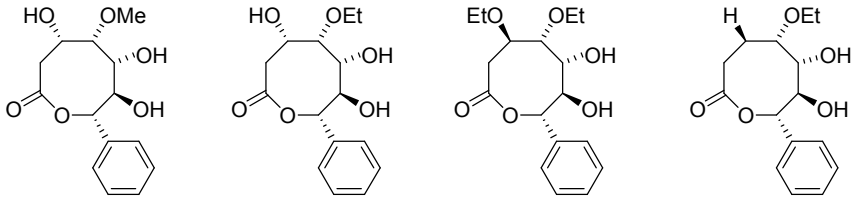
Two well known butenolide compounds are goniobutenolide-A (**16**), and goniobutenolide-B (**17**). They were originally isolated from *G. Giganteus* (Bermejo, et al. 1999). Biological activity of the compound **16** was tested against four different tumour cell lines (HL-60, HCT-8, MDA/MB-435 and SF295) by Teixeira research group and it has no effect on cell lines HL-60, MDA/MB-435 and SF295, but for the cell line HCT-8 a modest cytotoxicity was observed (IC_{50} = 101.5 μM) (Figure 2.6) (Teixeira, et al. 2007).

16 17

Figure 2.6. Structures of goniobutenolide-A (**16**), and goniobutenolide-B (**17**).

2.5. Heptolides

Gonioheptolides-A (**18**) and -B (**19**) isolated from the stem bark of *G. giganteus*, were the first compounds of this class. Recently two novel heptolides, almuheptolides-A (**20**) and -B (**21**), have been isolated from the stem bark of *G. arvensis*. Compounds of this group contain an unusual, saturated eight-membered lactone moiety (Figure 2.7) (Bermejo, et al. 1999).



18 19 20 21

Figure 2.7. Structures of gonioheptolides-A (**18**), gonioheptolides-B (**19**), almuheptolides-A (**20**) and -B (**21**).

2.6. Styryl-pyrones

The styryl-lactones make up an interesting group from the pharmacological point of view and styryl-pyrones are one of the interesting members of this group. They can be classified in four class depending the degree of oxidation of their aliphatic chain and in the saturation of the pyrone moiety. Goniothalamin (**1**), Goniodiol (**2**), Etharvendiol (**22**) are some members of this group (Figure 2.1 and 2.8) (Bermejo, et al. 1999).

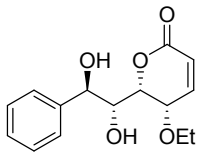


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Figure 2.8. Structure of etharvendiol (**22**).

Among styryl lactones family, goniothalamin (**1**) is especially interesting because of its selective cytotoxicity against breast cancer cell lines (Fatima, et al. 2006). Goniothalamin (**1**), a naturally occurring styryl lactone was isolated in 1967 from the dried bark of *Cryptocarya caloneura* (Pospisil and Marco 2006).

Later it was isolated from *Cryptocarya moschata*, and various species of *Goniothalamus* (Fatima and Pilli 2003). Addition to its cytotoxicity, goniothalamin (**1**) shows a potent mosquito larvicide, weak antibacterial, and significant antifungal activity against a wide range of gram-positive and gram-negative bacteria and fungi (Mosaddik and Haque 2003).

Recent studies have demonstrated that (R)-goniothalamin (**23**) has cytotoxic effects in vitro especially by inducing apoptosis on different cancer cell lines [cervical carcinoma (Hela), gastric carcinoma (HGC-27), breast carcinoma (MCF-7, T47D, and MDA-MB-231), leukemia carcinoma (HL-60), and ovarian carcinoma (Caov-3)]. The most interesting property of the R-goniothalamin (**23**) is, its minimal cytotoxicity against non-malignant cells (Figure 2.9) (Fatima, et al. 2005).

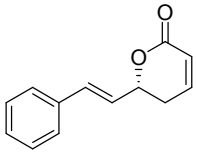

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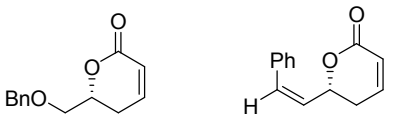
Figure 2.9. Structure of R-goniothalamin (**23**).

2.6.1. Structure Activity Relationship (SAR) of Goniothalamin Derivatives

Synthesis of R-goniothalamin (**23**) and preparation of the derivatives have gained importance in the last decade. During the synthesis of **23** Fatima, et al. has reported the cytotoxic properties of an intermediate **24** and a side product **25**. Compounds **24** and **25** were tested against different human cancer cell lines such as MCF-7 (breast), NCI-ADR (breast expressing the multidrug resistance phenotype), NCI 460 (lung, non-small cells), UACC62 (melanoma), 786-0 (kidney), OVCAR03 (ovarian), PC- 3 (prostate), and HT-29 (colon) (Figure 2.10).



24 25

Figure 2.10. Structures of linker modified analogues of goniothalamin (**24**-**25**).

All compounds displayed antiproliferative activity against tested cancer cell lines. R-goniothalamin (**23**) was found to be more potent than **24** and **25** toward [NCI-ADR (breast expressing the multidrug resistance phenotype), NCI 460 (lung, non-small cells), UACC62 (melanoma), 786–0 (kidney), and HT-29 (colon)]. Compound **24** and **25** were more cytotoxic than **23** towards prostate (PC-3), breast (MCF-7), and ovarian (OVCAR03) cancer cell lines (Fatima, et al. 2005). These results indicates that the trans oriented double bond in the linker sub-unit is important for the cytotoxicity in most of the tested cancer cell lines.

Fatima, et al. also published a study on synthesis and biological activities of goniothalamin (**23**) and its enantiomer **26**. The results of the study showed that both enantiomers **23** and **26** displayed antiproliferative activitiy against cancer cell lines. Both goniothalamin (**23**) and (**26**) displayed higher potency against 786-0 cell line (kidney tumor) and NCI.ADR (breast expressing the resistance phenotype for adryamycin) than DOX which one is used for positive control, more impressive results were obtained with the breast resistant cancer cell line (NCI.ADR), **23** showed to be 10 and 20 times more powerful than **26** and DOX. Respectively, for kidney cells (786-0),

26 was found to be 1,600-fold more potent than **23**. (IC_{50} for **26** is 4 nM, IC_{50} for **23** is 6.4 μM).

Cytotoxicity test results of **26** directed them to synthesize new (S)-goniothalamin analogues **27**-**32**. By these synthetic derivatives, they investigated the possible pharmocophoric groups responsible for the activity of goniothalamin (Figure 2.11). Also, they investigated the activity of the compounds which of the double bond of the pyranone ring was saturated **33**-**34** (Figure 2.12).

Test results were indicating that the endo and exo double bonds in the pyranone ring are essential for the activity of **26** against kidney cancer cell line (786-0). The analogues **27**, **33**, and **34** which are lack of either one or both double bonds, shows antiproliferative activity lower than that of **26**. Also, the importance of the E-configuration of the styryl part in **23** for antiproliferative activity was explained, it is tought to be role of Michael acceptor in the pyranone ring for nucleophilic amino acid residues present in the natural receptors, which are possibly interacting with these compounds (Fatima, et al. 2006).

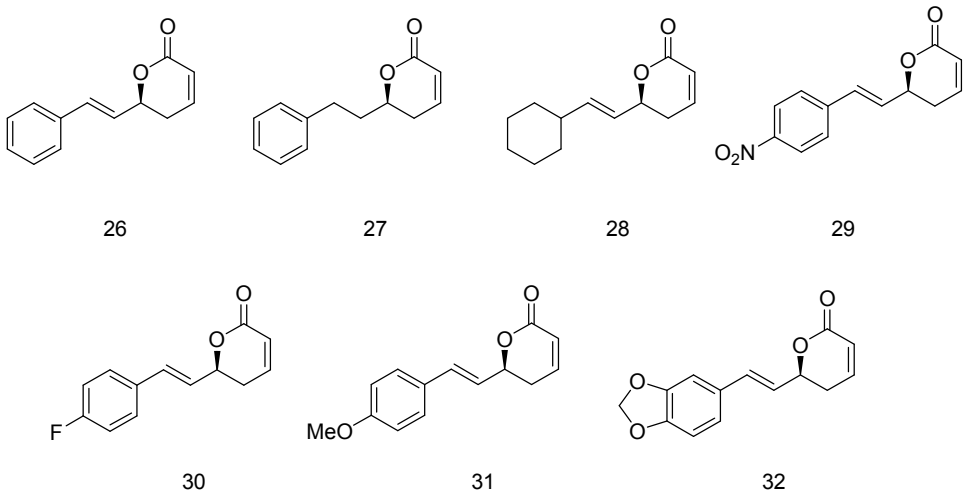
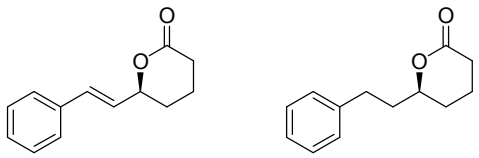


Figure 2.11. Structures of (S)-goniothalamin (**26**) and its analogues **27**-**32**.

Moreover, for analogues **29** to **32** the results obtained from the cytotoxicity test against kidney cancer cells demonstrated that electron-donating or electron-withdrawing groups in the aromatic ring decreased their potency compared to **26** and steric hinderance may play role in this diminished cytotoxicity.

Antiproliferative activities of analogues differentiating, such as **23** was found to be more potent than **26** against the melanoma cell line (UACC.62), but the analogues **28**, **31** and **33** were 7-, 3- and 3-fold more potent than **23** relatively. Similarly, analogues **28** and **31** were found to be more potent than **26** and **23** against prostate cancer cell (PC-3) proliferation (Fatima, et al. 2006).

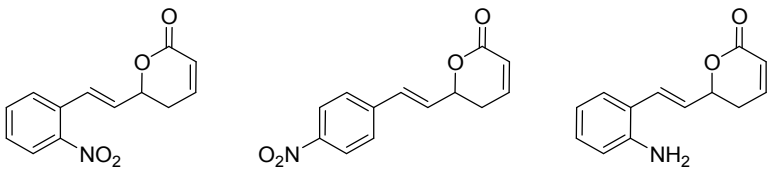


33 34

Figure 2.12. Structures of (S)-goniothalamin analogues having saturated lactone rings **33-34**.

In addition, Zhou, et al. studied the semisynthesis and antitumor activities of new goniothalamin derivatives **35-46**, and cytotoxic activity tests were performed against human promyelocytic leukemia (HL-60), human hepatoma (BEL-7402), human lung carcinoma (A549) and human stomach cancer (SGC-7901) cell lines.

Semi-synthetic styryl lactone derivatives **35**, **36** and **37** showing stronger inhibitory effect against the human stomach tumor SGC-7901. Additional derivatives (**34-36**), having free amino and acylamino substituent at position 10, have also been prepared and tested for cytotoxicity. The results are showing that IC_{50} values of the acylamino compounds increased for A549 and SGC-7901. It was proposed that free amino group at C-10 is important for antitumor activity, and activity get lost when the lone pairs of electrons on nitrogen were used for resonance with carbonyl group. They also investigated the antitumor activities of amino acid functionalized derivatives. Biological activity tests resulted that amino acid group derivatives of goniothalamin could not enhance the activity as compound **37** does (Figure 2.13) (Zhou, et al. 2005).



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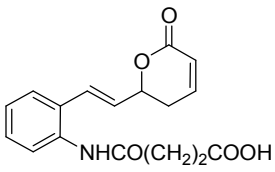

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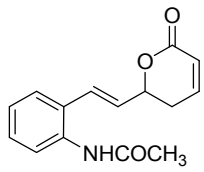

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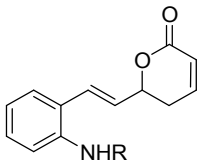
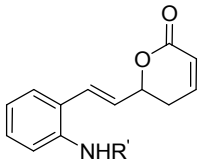


40-43



44-46

40, R=NHBoc-L-Gly

41, R=NHBoc-L-Ala

42, R=NHBoc-L-Val

43, R=NHBoc-L-Phe

44, R'=L-Ala

45, R'=L-Leu

46, R'=L-Ile

Figure 2.13. Structures of *o*-nitro **35**, *p*-nitro **36**, *o*-amino **37**, *o*-amide **38**-**39** and *o*-amino acid substituted goniothalamin derivatives **40**-**46**.

As it is summarized above, cytotoxic activity studies of goniothalamin (**1**) derivatives have been studied mostly in last decades, and four essential part of this molecule has been modified in these works. The sub-units of goniothalamin has been represented in Figure 2.14. As indicated above, the results of these SAR studies of goniothalamin derivatives imply that, Michael acceptor sub-unit in the lactone ring, trans oriented double bond in the linker part, and configuration of the stereogenic carbon play an important role in the cytotoxic activity.

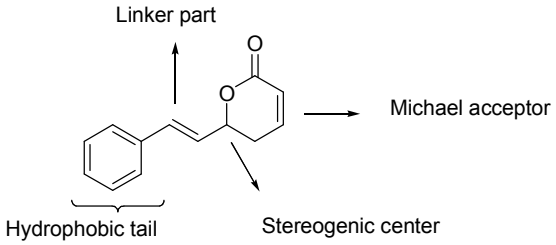


Figure 2.14. Sources of the activity in goniothalamin (**1**) structure.

In summary, the most essential sub-unit of the goniothalamin is α,β-unsaturated lactone ring actually. The role of the rest of the molecule seems still questionable because of the observation of different responses of the cancer cell lines to the derivatives of **23** and **26**. Any change in the linker part, stereogenic center or hyrophobic tail mostly returned with moderest cytotoxic difference, and for few cases a high activity toward one type of cancer cell line.

CHAPTER 3

SYNTESIS OF GONIOTHALAMIN DERIVATIVES

3.1. Synthesis in Literature

Due to the interesting biological activities of goniothalamin (**1**) derivatives, several successful synthesis has been described in literature (Fatima, et al. 2006).

Racemic Goniothalamin (**1**) synthesis was performed by Fournier, et al., by starting from trans-cinnamaldehyde (**49**). A short retrosynthetic analysis was shown in Figure 3.1. The synthesis of goniothalamin (**1**) was performed through the formation of a β-lactone intermediate (**47**) (Fournier, et al. 2004).

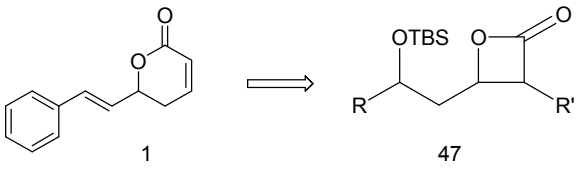


Figure 3.1. Retrosynthetic analysis of goniothalamin (**1**).

Because of the existence of a stereogenic center in goniothalamin structure, it is essential to synthesize both enantiomers by enantioselective fashion to observe the dependence of activity with the configuration at stereogenic center.

As shown in Figure 3.2, different starting points have been chosen from the literature for these asymmetric syntheses of R-goniothalamin (**23**) or S-goniothalamin (**26**). In these strategies, either an enantiomerically pure starting material was chosen (Pospisil and Marko 2006) or an enzymatic kinetic transesterification of racemic homoallylic alcohol **51** was performed (Sundby, et al. 2004, Gruttadauria, et al. 2004) from propargylic alcohol **48** or the stereogenic center was installed by a catalytic asymmetric synthesis starting from aldehydes **49**, and **52** (Sabitha, et al. 2006, Ramachandran, et al. 2006, Fatima, et al. 2005, Fatima, et al. 2006).

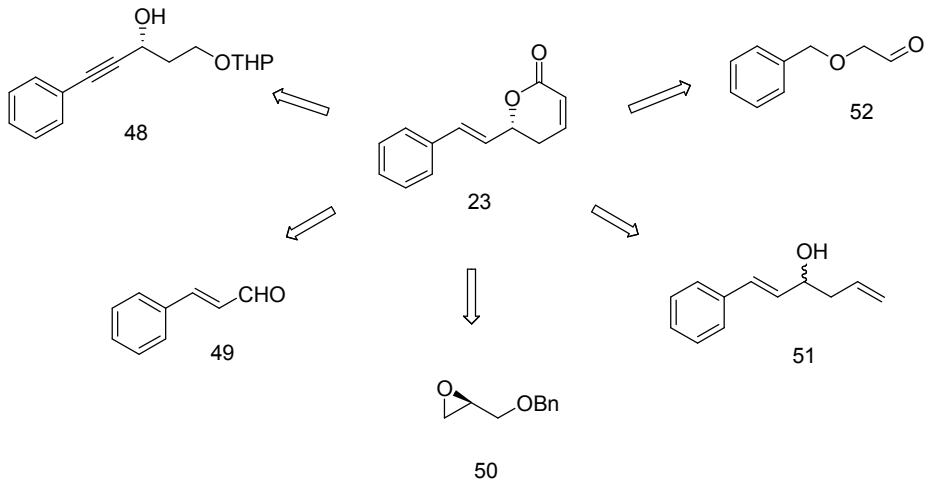


Figure 3.2. Retrosynthetic analysis of asymmetric synthesis of goniothalamin derivatives.

There are also some semisynthesis procedures represented in literature. Zhou,, et al. represented a study on the preparation of derivatives **35**-**44** starting from naturally occuring goniothalamin (**1**). Key point of these modifications was the nitration of aromatic phenyl ring. Then it would be further reduced to amino derivative **36** and then easily be converted to amides **38**-**44** (Figure 3.3) (Zhou, et al. 2005).

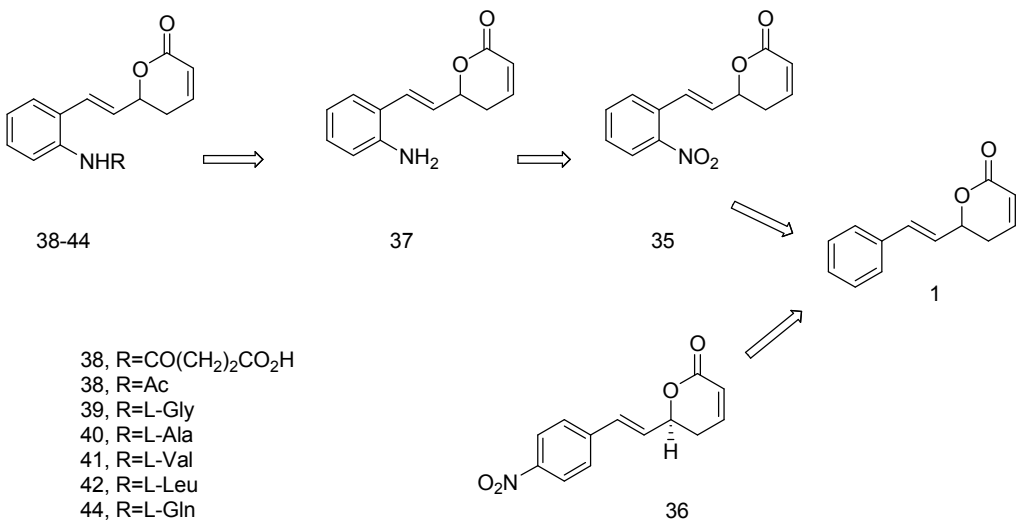


Figure 3.3. Retrosynthetic analysis of semisynthesis of goniothalamin derivatives.

Because of the asymmetric synthesis of goniothalamin derivatives is a subject of this study, detailed explanation of these reactions should be dicussed.

3.2. Lipase Catalyzed Resolution and Alkene Metathesis

Sundby, et al. was reported the combination of enzymatic trans esterification and alkene metathesis in two steps. In this synthesis method, firstly racemic alcohol **51** was prepared by Grignard reaction between allylmagnesium bromide and *trans*-cinnamaldehyde (**49**). Then it was kinetically resolved by a transesterification reaction in hexane using vinyl acrylate as acryl donor and *Candida antarctica* lipase B (CALB) as catalyst and compounds **53-54** were obtained. After separation of the products, alcohol **54** was then treated with acryloyl chloride and finally obtained acrylates **53** and **55** were treated with Grubbs' catalyst to give (S)- and (R)-goniothalamin (Figure 3.4) (Sundby, et al. 2004).

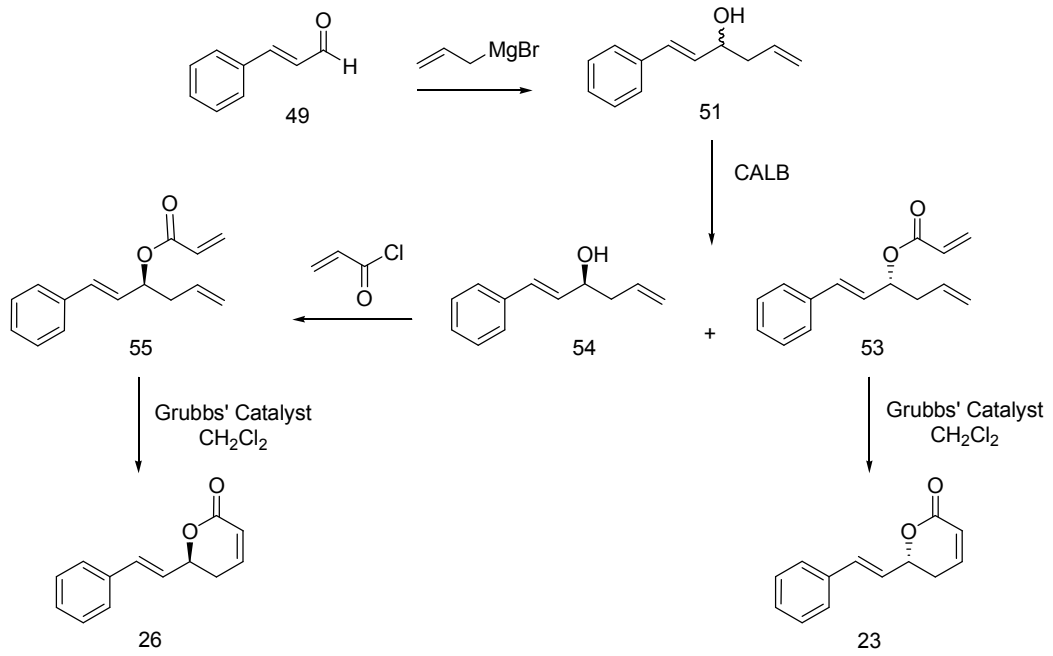


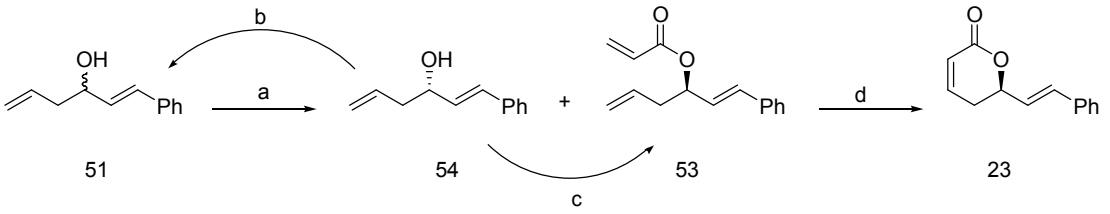
Figure 3.4. Lipaze catalyzed synthesis of (S)- and (R)-goniothalamin (**26** and **23**).

3.3. Chemoenzymatic Synthesis

Lipases are the most widely employed enzymes because they are cheap and readily available from many different sources, and in addition, they possess high enantioselectivity for a broad range of substrates and high stability in organic solvents (Gruttadauria, et al. 2004).

Gruttadauria, et al. reported the three step synthesis of goniothalamin by an enzymatic kinetic resolution in the presence of vinyl acrylate followed by ring closing metathesis.

In this study, starting material was the racemic allylic alcohol **51** was used and the resolution was carried out with PS-C Amano II to form the ester of one enantiomer. After the purification of this ester **53**, olefin metathesis was performed with the Grubbs' catalyst. In this study to improve the yield, they racemized alcohol **54** back **51**. The starting racemate was achieved in one pot synthesis by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in diethyl ether and then adding methanol and $NaBH_4$ (Figure 3.5).



Reagents and conditions: (a) PS-C Amano II, solvent, vinyl acrylate, 25 ^oC, 24 h; (b) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), diethyl ether, then CH_3OH, $NaBH_4$, rt; (c) PPh_3, DEAD, acrylic acid, toluene, 0 ^oC–rt 21%, 20% ee; (d) $(Pcy_3)_2Cl_2Ru$=CHPh, $Ti(OiPr)_4$, CH_2Cl_2, 18 h, reflux.

Figure 3.5. Asymmetric synthesis of (R)-goniothalamin (**23**) with chemoenzymatic synthesis.

3.4. Asymmetric Allylboration

In an enantioselective synthesis, one of the fundamental C-C bond forming reaction is, catalytic enantioselective allylation of aldehydes with chiral catalyst.

Gruttadauria, et al. reported a study on asymmetric synthesis of R-goniothalamin (**23**). In this route synthesis was started with the allylboration of *trans*-cinnamaldehyde (**49**) with (+)-*B*-allyldiisopinocampheylborane (**56**) in an diethylether-pentane mixture at -100 °C to gave homoallylic alcohol **57** with 72% yield. Esterification of **57** with acryloyl chloride **58** in the presence of a base provided corresponding acryloyl ester **53**, which then treated with Grubbs' catalyst in refluxing CH_2Cl_2 for 6 h to yield **23**. Final product was obtained with 76% yield and 92% ee. (Figure 3.6) (Gruttadauria, et al. 2004).

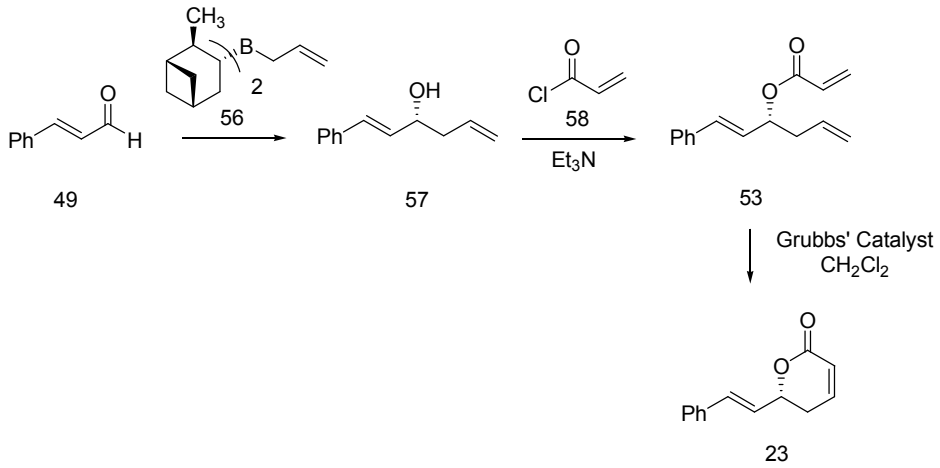


Figure 3.6. Asymmetric synthesis of (R)-goniothalamin (**23**) starting with allylboration with (+)-*B*-allyldiisopinocampheylborane (**56**).

3.5. Asymmetric Allylation of Aldehydes

Another three step total synthesis of (*R*)-goniothalamin (**23**) was also described by Fatima, et al. The first step was the catalytic asymmetric allylation of an aldehyde with allyltributyltin under the influence of chiral catalyst **60** which can be prepared from (R)-BINOL (**59**) in situ (Figure 3.7). The μ-oxobis(binaphthoxy)(isopropoxy)-titanium complex displays excellent enantioselectivity for the addition of allyltributyltin to aldehydes. The efficiency of this catalyst is resulting from the simultaneous coordination and double activation ability of the bidentate Ti (IV) catalyst (Fatima, et al. 2003). This catalyst was developed by Maruoka and coworkers provided higher level of enantioselection (Mauroka, et al. 2003).

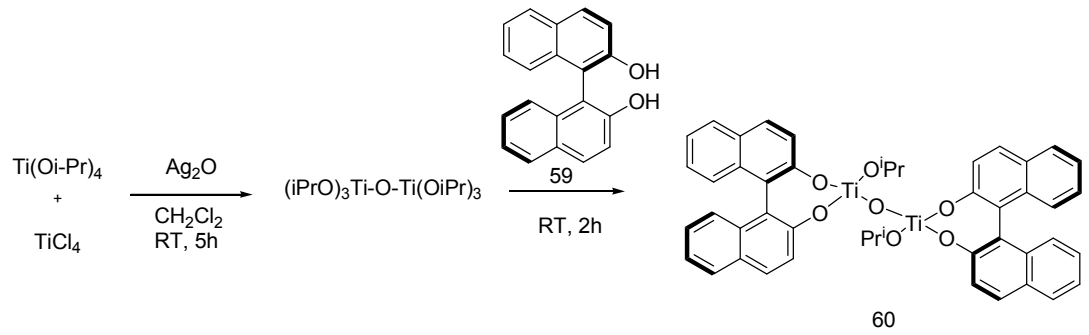
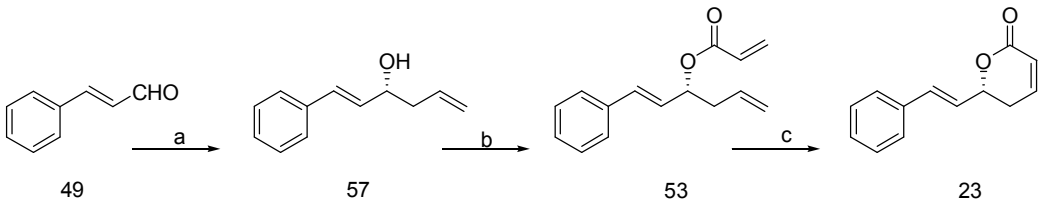


Figure 3.7. Preparation of chiral auxiliary **60** from (R)-BINOL (**59**) in situ.

After the preparation of **60** it was used in the catalytic asymmetric allylation of *trans*-cinnamaldehyde (**49**) with allyltributyltin (**61**) to produce allyl alcohol **57** with a moderate yield (78%) and good enantioselectivity (96% ee). In the next step resulting homoallylic alcohol **57** was treated with acryloyl chloride (**58**) to produce acryloyl ester **54** in the presence of triethylamine with 80% yield. Finally, ring closing metathesis was performed with Grubbs' catalyst to produce **23** (Figure 3.8). Transformation of *trans*-cinnamaldehyde (**49**) into **23** with 61% overall yield. This is the most efficient approach so far reported in the literature for these natural product and illustrates the utility of the asymmetric catalytic allylation protocol developed by Maruoka and co-workers.



Reagents and conditions: (a) (*R*)-BINOL (10 mol%), $Ti(OiPr)_4$ (15 mol%), $TiCl_4$ (5 mol%), Ag_2O (10 mol%), allyltributyltin (1.1. equiv.), CH_2Cl_2, −20°C, 24 h (78%; 96% ee); (b) acryloyl chloride (1.8 equiv.), Et_3N (3.6 equiv.), CH_2Cl_2, 0°C (80%); (c) Grubbs' catalyst $[(Pcy_3)_2Cl_2Ru{=}CHPh]$ (10 mol%), CH_2Cl_2 (98%).

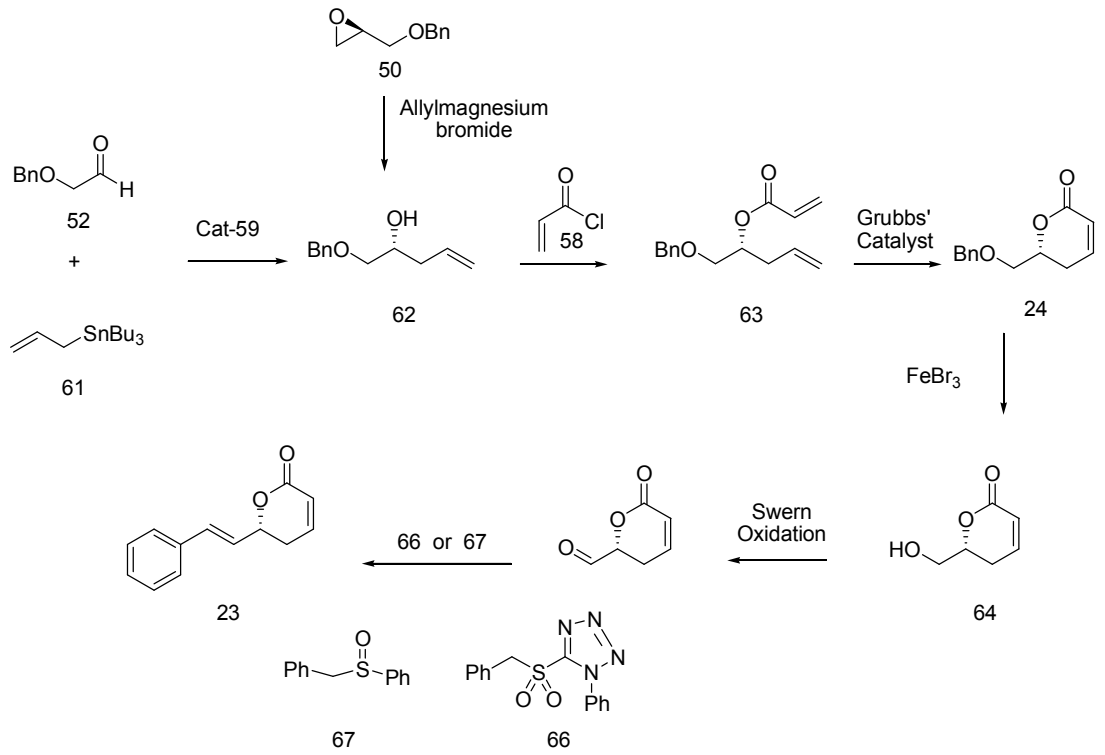
Figure 3.8. Asymmetric synthesis of (R)-goniothalamin (**23**) starting with asymmetric allylation of *trans*-cinnamaldehyde.

3.6. Sulfoxide-Modified Julia Olefination

In literature, different sulfoxide modified olefination methods represented for the application of enantioselective R-goniothalamin (**23**) synthesis. Fatima, et al. and Pospisil-Marco's research groups represented two different pathways for this synthesis. First synthesis was accomplished by Fatima, et al. and they performed the asymmetric catalytic allylation protocol to benzyloxyacetaldehyde (**52**) with allytributyltin (**61**) to form allylic alcohol **62**. This obtained allylic alcohol **62** was then acrylated to yield ester **63** which was treated with Grubbs' catalyst to give lactone product **24**. Deprotection of benzyl group in compound **24** was performed in the presence of $FeCl_3$ and gave the alcohol **64**. Swern oxidation of **64** yielded the unstable aldehyde **65**, which was immediately reacted with sulfoxide **66** under suitable reaction conditions to accomplish the desired olefination with 13% yield (Figure 3.9) (Fatima, et al. 2003).

A similar synthesis was performed by Pospisil and Marco, they started synthesis with glycidol ether **50** by a ring opening reaction with an allyl group, acrylation, and metathesis sequences were followed. Deprotection of the alcohol was performed in the same way by Fatima, et al., then obtained unstable aldehyde **65** was immediately reacted with benzylic sulfoxide **67** under standard sulfoxide-modified Julia olefination sequence, developed by them, to produce (R)-goniothalamin (**23**) with 78% yield.

Also in this study, they noted that sulfoxide-modified Julia olefination afforded the natural product **23** with both an excellent yield and nearly perfect control of the C6–C7 double bond geometry, contrast to the results obtained using alternative olefination methods, such as Wittig, classical Julia and Kociensky–Julia protocols (Pospisil and Marco 2006).



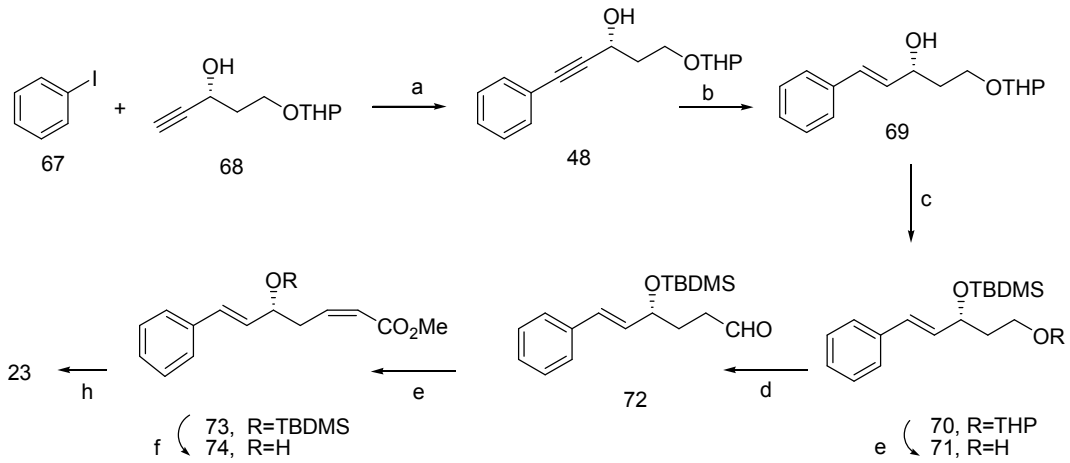
Reagents and Conditions for Fatima, et al. Synthesis Method: (a) (R)-BINOL (10 mol %), Ti(O*i*-Pr)$_4$ (10 mol %), molecular sieves (4 Å), allyltributyltin, -20 ^oC, 60 h (78%; 94% ee); (b) acryloyl chloride, Et_3N, CH_2Cl_2, 0 ^oC (86%); (c) Grubbs' catalyst [$(PCy_3)_2Cl_2$ Ru=CHPh], CH_2Cl_2 (91%); (d) $FeCl_3$, CH_2Cl_2 (88%); (e) $(COCl)_2$, CH_2Cl_2, DMSO, Et_3N, -65 ^oC, 30 min; then Ph_3P=CHPh, THF (53%, two steps); (f) $(COCl)_2$, CH_2Cl_2, DMSO, Et_3N, -65 ^oC, 30 min; then solution of the 66, KHMDS, THF, -78 ^oC (<20%).

Sulfoxide Modified Julia-olefination Reagents and conditions: (1) LDA (1.1 eq), benzylic sulfoxide 67 (1 eq.), THF, -78^oC, 30min; (2) Aldehyde (62) (1.05 eq.), -78 ^oC, 2h; (3) BzCl (1.5 eq.), -78 ^oC to r.t; (4) $Me_2N(CH_2)_3NH_2$ (1.55 eq.); (5) SmI_2 (4eq.), HMPA (4 eq.) THF, -78^oC, 30 min.

Figure 3.9. Asymmetric synthesis of (R)-goniothalamin (**23**) with sulfoxide-modified Julia olefination.

3.7. Crosford Cross-Coupling Protocol

Another stereoselective synthesis of R-goniothalamin (**23**) was reported by Sabitha, et al., who developed a method which involves the Crosford cross-coupling protocol, starting from the reaction of iodobenzene (**67**) and an acetylenic alcohol **68**.



Reagents and conditions: (a) 10% Pd/C (cat.), CuI (4 cat.), Ph_3P (0.1 equiv), K_2CO_3, H_2O/DME, 80°C, 2 h, 90%. (b) $LiAlH_4$, THF, 0°C to rt, 2 h, 90%; (c) TBDMSCl, imidazole, DCM, DMAP, 2 h, 95%; (d) PPTS, MeOH, 12 h, 90 %; (e) IBX, DMSO, DCM, 0°C to rt, 2 h, 75%; (f) i. NaH/THF, -78°C, 30 min; ii. $(CF_3CH_2O)_2P(O)CH_2COOCH_3$, THF, -70°C, 30 min, 80%; (g) TBAF, THF, 2 h, 80%; (h) benzene, reflux, PTSA, 1 h, 75%.

Figure 3.10. Asymmetric synthesis of (R)-goniothalamin (**23**) with Crosford cross-coupling protocol.

The product of this reaction, compound **48**, plays a key role in the stereoselective synthesis of various natural products. Resulting propargylic alcohol **48** was reduced with LiAH in THF to form compound **69**. The secondary hydroxyl group was silylated with TBDMSCl to provide **70** and subsequently the primary THP group was cleaved using PPTS in MeOH to afford compound **71**. The alcohol was subjected to oxidation in the presence of IBX in DCM to furnish the aldehyde **72**. Resulting aldehyde was converted to an α,β-unsaturated ester **73**. Deprotection of TBDMS followed by lactonization gave **23** (Figure 3.10) (Sabitha, et al. 2006).

CHAPTER 4

EXPERIMENTAL

4.1. Organic Chemistry Part

4.1.1. General Methods

Reagents were commercial grade and were used as supplied. Dichloromethane was distilled over calcium hydride. Reactions were monitored by TLC chromatography using Merck TLC plates (Silicagel 60 F_{254}). Chromatographic separations were performed using 70–230 mesh silicagel. Solvents, required for SiO_2 column chromatography, were commercial grade and were used as supplied. Solvents, required for HPLC, were spectrometric grade and were used as supplied. ^{1}H NMR and ^{13}C NMR data were recorded on a Varian 400-MR (400 MHz) spectrometer. Chemical shifts for ^{1}H-NMR and ^{13}C-NMR are reported in δ (ppm). $CDCl_3$ peaks were used as reference in ^{1}H-NMR (7.26 ppm), and ^{13}C-NMR (77.36 ppm) respectively. Optical rotations were measured with Optical Digital Polarimeter (SOLF) model WZZ-1S instrument. HPLC studies were performed by employing Chiracel AD-H column (0,46x150 mm) on Agilent 1100 Series HPLC. GC-Mass spectra (EI) were measured on Agilent 6890N Network GC System equipped with a Quadrupole Mass Spectrometer (EI).

4.1.2. Preparation of (R)-goniothalamin (23)

Racemic goniothalamin (**1**) was prepared according to the literature, defined by Fatima, et al. in the absence of R-BINOL (**59**) with 36% overall yield. Product was purified from SiO_2 column and enantiomers were monitored in HPLC Chiracel AD-H Column, (*i*-propanol:hexane (1:9), 1 mL/min t_1=7.65 min. and t_2=7.98 min.). Then R-Goniothalamin (**23**) was synthesized by same procedure in the presence of R-BINOL starting from trans-cinnamaldehyde with 38% overall yield and 65% ee. Enantiomers were monitored in HPLC Chiracel AD-H Column, with the same conditions and it was

seen that the ratio of the peaks was different than that of racemic one and enantiomeric excess was calculated by using the area under these signals.

4.1.3. Preparation of R-(+)-1-(naphthalen-2-yl)-but-3-en-1-ol (78)

In a 10 mL round-bottom flask equipped with a magnetic stirring bar and a condenser, 25 mg (14 µL, 0.13 mmol) of $TiCl_4$ was dissolved in 2.6 mL of dichloromethane. To this solution, 118 mg (120 µL, 0.41 mmol) of Ti(O*i*-Pr)$_4$ was added under nitrogen atmosphere. The resulting solution was allowed to warm up to room temperature for 1 h and then 61 mg (0.26 mmol) of silver (I) oxide was added. The mixture was stirred for 5 h without any exposure to direct day light. After dilution with 4.5 mL of dichloromethane, 150 mg (0.52 mmol) of (R)-BINOL (**59**) was added and stirred for 2 h. The resulting mixture was cooled down to -75 ^{o}C, and then treated sequentially with 409 mg (2.62 mmol) of 2-naphthaldehyde (**77**) and 953 mg (893 µL, 2.88 mmol) of allyltributyltin (**61**). The mixture was stirred for 16 h and allowed to warm up to room temperature. After the reaction was completed, the reaction mixture was filtered through a short pad of celite, then quenched with saturated $NaHCO_3$ solution, and extracted with 3x30 mL of ethyl acetate. The organic extracts were combined and dried over $MgSO_4$. After the removal of solvent under vacuum, purification of the crude by column chromatography on silica gel (ethyl acetate:hexanes, 1:8) furnished 309 mg of R-(+)-1-(naphthalen-2-yl)-but-3-en-1-ol (**78**) as a colorless foam with 59% yield. R_f = 0.65 (ethyl acetate:hexanes, 1:2); $[\alpha]_D^{30}$ = + 52.7^{o} (c = 3, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.88-7.78 (m, 4H), 7.53-7.44 (m, 3H), 5.91-5.77 (m, 1H), 5.23-5.12 (m, 2H), 4.90 (t, 1H, J = 6.3 Hz), 2.68-2,54 (m, 2H), 2.25 (s, 1H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 141,58, 134.68, 133.59, 133.28, 128.52, 128.28, 128.00, 126.44, 126.13, 124.83, 124.33, 118.81, 73.72, 44.04; MS (EI) *m/z* calculated for M^+ ($C_{14}H_{14}O$) = 198,1; found: 198 (2%), 179 (100%), 166, 157, 129; Enantiomeric excess was found as 83% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 10:90, 1mL/min t_1 = 5.51 min "major enantiomer", t_2 = 5.97 min "minor enantiomer").

4.1.4. Preparation of R-(+)-1-(naphthalen-2-yl)-but-3-enyl acrylate (79)

A solution of 302 mg (1.52 mmol) of **78** in 3.0 mL of dichloromethane was cooled down to 0 ^{o}C; then 248 mg (223 μL, 2.74 mmol) of acryloyl chloride (**58**) and 555 mg (770 μL, 5.48 mmol) of triethyl amine were sequentially added. The mixture was warmed to room temperature and stirred for 22 h under nitrogen atmosphere. The resulting mixture was filtered through a short pad of celite, poured into water, and the product was extracted with 3x25 mL of dichloromethane. The combined organic layer was concentrated under reduced pressure and purification of this residue by column chromatography on silica gel (ethyl acetate:hexane, 1:8) gave 205 mg of R-(+)-1-(naphthalen-2-yl)-but-3-enyl acrylate (**79**) with 53% yield. R_f = 0.63 (ethyl acetate:hexanes, 1:4); $[\alpha]_D^{25}$ = + 67.46^{o} (c = 2.05, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.88-7.70 (m, 4H), 7.53-7.41 (s, 3H), 6.44 (d, 1H, J = 17.3 Hz), 6.24-6.12 (m, 1H), 6.09-6.00 (m, 1H), 5.84 (d, 1H, J = 10.4 Hz), 5.80-5.67 (m, 1H), 5.16-5.00 (m, 2H), 2.85-2.63 (m, 2H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 165.74, 137.64, 133.49, 133.45, 133.44, 131.28, 128.92, 128.65, 128.41, 128.01, 126.56, 126.45, 126.10, 124.62, 118.56, 75.86, 41.00;MS (EI) *m/z* calculated for M^+ ($C_{17}H_{16}O_2$) = 252,1; found: 252 (2%), 224, 178, 156(100%), 128, 68; Enantiomeric excess was found as 79% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 10:90, 1mL/min t_1 = 2,80 min "major enantiomer", t_2 = 3,50 min "minor enantiomer").

4.1.5. Preparation of R-(+)-6-(naphthalen-2-yl)-5,6-dihydro-2H-pyran-2-one (75)

To a stirred solution of 80 mg (0.1 mmol) of Grubbs' catalyst (10 mol%) in 8 mL dichloromethane at 60 ^{o}C was added a solution of 205 mg (0.81 mmol) of **79** in 90 mL of dichloromethane. The resulting mixture was heated for 14 h. At the end of this period, the mixture was cooled to room temperature and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (ethyl acetate:hexanes, 1:8) furnished 159 mg R-(+)-6-(naphthalen-2-yl)-5,6-dihydro-2H-pyran-2-one (**75**) with 88% yield. R_f=0.13 (ethyl acetate:hexanes, 1:4). $[\alpha]_D^{20}$=+ 190.19^{o} (c = 1.59, CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.91-7.82 (m, 4H), 7.54-7.47 (m, 3H), 7.03-6.96 (m, 1H), 6.18 (d, 1H, J = 10.9 Hz), 5.63 (dd, 1H, J = 10.7, and 5.2 Hz),

2.80-2.64 (m, 2H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 164.41, 145.19, 136.13, 133.59, 133.42, 128.46, 128.08, 126.87, 126.83, 125.53, 123.86, 122.13, 105.11, 79.65, 32.08; MS (EI) *m/z* calculated for M^+ ($C_{15}H_{12}O_2$) = 224,1; found: 224 (50%), 178, 156 (100%), 128, 68; Enantiomeric excess was found as 76% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 5:95, 1mL/min t_1 = 17,66 min, "major enantiomer", t_2 = 18.27 min "minor enantiomer")

4.1.6. Preparation of S-(-)-1-(naphthalen-2-yl)-but-3-en-1-ol (80)

In a 10 mL round-bottom flask equipped with a magnetic stirring bar and a condenser, 25 mg (14 μL, 0.13 mmol) of $TiCl_4$ was dissolved in 2.6 mL of dichloromethane. To this solution, 118 mg (120 μL, 0.41 mmol) of Ti(O*i*-Pr)$_4$ was added under nitrogen atmosphere. The resulting solution was allowed to warm up to room temperature for 1 h and then 61 mg (0.26 mmol) of silver (I) oxide was added. The mixture was stirred for 5 h without any exposure to direct day light. After dilution with 4.5 mL of dichloromethane, 150 mg (0.52 mmol) of (S)-BINOL was added and stirred for 2 h. The resulting mixture was cooled down to -75 ^oC, and then treated sequentially with 409 mg (2.62 mmol) of 2-naphthaldehyde (**77**) and 953 mg (893 μL, 2.88 mmol) of allyltributyltin (**61**). The mixture was stirred for 18 h and allowed to warm up to room temperature. After the reaction was completed, the reaction mixture was filtered through a short pad of celite, then quenched with saturated $NaHCO_3$ solution, and extracted with 3x30 mL of ethyl acetate. The organic extracts were combined and dried over $MgSO_4$. After the removal of solvent under vacuum, purification of the crude by column chromatography on silica gel (ethyl acetate:hexanes, 1:8) furnished 133 mg of S-(-)-1-(naphthalen-2-yl)-but-3-en-1-ol (**80**) as a light yellow solid with 26% yield. R_f = 0.42 (ethyl acetate:hexanes, 1:4); $[\alpha]_D^{26}$= -47.87^o (c = 1.32, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.88-7.78 (m, 4H), 7.53-7.44 (m, 3H), 5.91-5.77 (m, 1H), 5.23-5.12 (m, 2H), 4.90 (t, 1H, J = 6.3 Hz), 2.68-2,54 (m, 2H), 2.25 (s, 1H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 141,58, 134.68, 133.59, 133.28, 128.52, 128.28, 128.00, 126.44, 126.13, 124.83, 124.33, 118.81, 73.72, 44.04; MS (EI) *m/z* calculated for M^+ ($C_{14}H_{14}O$) = 198,1; found: 198 (2%), 179 (100%), 166, 157, 129; Enantiomeric excess was found as 81% with HPLC - Chiracel AD-H column

(*i*-propanol:hexane 10:90, 1mL/min t_1 = 5.51 min "R enantiomer", t_2 = 5.95 min "S enantiomer").

4.1.7. Preparation of S-(-)-1-(naphthalen-2-yl)-but-3-enyl acrylate (81)

A solution of 133 mg (0.49 mmol) of **80** in 3.0 mL of dichloromethane was cooled down to 0 ^oC; then 100 mg (90 μL, 1.12 mmol) of acryloyl chloride (**58**) and 225 mg (312 μL, 2.22 mmol) of triethyl amine were sequentially added. The mixture was warmed to room temperature and stirred for 3 h under nitrogen atmosphere. The resulting mixture was filtered through a short pad of celite, poured into water, and the product was extracted with 3x25 mL of dichloromethane. The combined organic layer was concentrated under reduced pressure and purification of this residue by column chromatography on silica gel (ethyl acetate:hexane, 1:8) gave 124 mg of S-(-)-1-(naphthalen-2-yl)-but-3-enyl acrylate (**81**) with 73% yield. R_f = 0.63 (ethyl acetate:hexanes, 1:4); $[\alpha]_D^{27}$ = -54.79^o (c = 1.23, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.88-7.70 (m, 4H), 7.53-7.41 (s, 3H), 6.44 (d, 1H, J = 17.3 Hz), 6.24-6.12 (m, 1H), 6.09-6.00 (m, 1H), 5.84 (d, 1H, J = 10.4 Hz), 5.80-5.67 (m, 1H), 5.16-5.00 (m, 2H), 2.85-2.63 (m, 2H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 165.74, 137.64, 133.49, 133.45, 133.44, 131.28, 128.92, 128.65, 128.41, 128.01, 126.56, 126.45, 126.10, 124.62, 118.56, 75.86, 41.00;MS (EI) *m/z* calculated for M^+ ($C_{17}H_{16}O_2$) = 252,1; found: 252 (2%), 224, 178, 156(100%), 128, 68; Enantiomeric excess was found as 81% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 5:95, 1mL/min t_1 = 3.21 min "S enantiomer", t_2 = 4.22 min "R enantiomer").

4.1.8. Preparation of S-(-)-6-(naphthalen-2-yl)-5,6-dihydro-2H-pyran-2-one (76)

To a stirred solution of 47 mg (0.06 mmol) of Grubbs' catalyst (10 mol%) in 4.5 mL dichloromethane at 60 ^oC was added a solution of 120 mg (0.48 mmol) of **81** in 53 mL of dichloromethane. The resulting mixture was heated for 16 h. At the end of this period, the mixture was cooled to room temperature and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (ethyl acetate:hexanes, 1:8) furnished 78 mg of S-(-)-6-(naphthalen-1-yl)-5,6-dihydro-2H-

pyran-2-one (**76**) as a colorless solid with 73% yield. R_f=0.13 (ethyl acetate:hexanes, 1:4). $[\alpha]_D^{20}$= -175.38^o (c = 0.78, CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.91-7.82 (m, 4H), 7.54-7.47 (m, 3H), 7.03-6.96 (m, 1H), 6.18 (d, 1H, J = 10.9 Hz), 5.63 (dd, 1H, J= 10.7, and 5.2 Hz), 2.80-2.64 (m, 2H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 164.41, 145.19, 136.13, 133.59, 133.42, 128.46, 128.08, 126.87, 126.83, 125.53, 123.86, 122.13, 105.11, 79.65, 32.08; MS (EI) *m/z* calculated for M^+ ($C_{15}H_{12}O_2$) = 224,1; found: 224 (50%), 178, 156 (100%), 128, 68; Enantiomeric excess was found as 43% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 5:95, 1mL/min t_1 = 17.52 min, "R enantiomer", t_2 = 18.25 min "S enantiomer")

4.1.9. Preparation of R-(+)-1-(naphthalen-1-yl)-but-3-en-1-ol (94)

In a 10 mL round-bottom flask equipped with a magnetic stirring bar and a condenser, 25 mg (14 µL, 0.13 mmol) of $TiCl_4$ was dissolved in 2.6 mL of dichloromethane. To this solution, 118 mg (120 µL, 0.41 mmol) of Ti(O*i*-Pr)$_4$ was added under nitrogen atmosphere. The resulting solution was allowed to warm up to room temperature for 1 h and then 61 mg (0.26 mmol) of silver (I) oxide was added. The mixture was stirred for 5 h without any exposure to direct day light. After dilution with 4.5 mL of dichloromethane, 150 mg (0.52 mmol) of (R)-BINOL (**59**) was added and stirred for 2 h. The resulting mixture was cooled down to -75 ^{o}C, and then treated sequentially with 409 mg (2.62 mmol) of 1-naphthaldehyde (**88**) and 953 mg (893 µL, 2.88 mmol) of allyltributyltin (**61**). The mixture was stirred for 16 h and allowed to warm up to room temperature. After the reaction was completed, the reaction mixture was filtered through a short pad of celite, then quenched with saturated $NaHCO_3$ solution, and extracted with 3x30 mL of ethyl acetate. The organic extracts were combined and dried over $MgSO_4$. After the removal of solvent under vacuum, purification of the crude by column chromatography on silica gel (ethyl acetate:hexanes, 1:8) furnished 214 mg of R-(+)-1-(naphthalen-1-yl)-but-3-en-1-ol (**94**) as a light yellow solid with 41% yield. R_f = 0.42 (ethyl acetate:hexanes, 1:4); $[\alpha]_D^{26}$ = + 84.35^o (c = 2.14, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.07 (d,1H, J=7.18 Hz), 7.92-7.87 (m,1H), 7.79 (d,1H, J=8.08 Hz), 7.67 (d,1H, 7.18 Hz), 7.57-7.46 (m,3H), 5.99-5.88 (m,1H), 5.55-5.48 (m,1H), 5.26-5.16 (m,2H), 2.81-2.73 (m,1H), 2.66-2.56 (m,1H), 2.37 (bs,1H); ^{13}C-NMR (400MHz, $CDCl_3$) δ 139.34, 134.71, 133.68, 130.15,

128.87, 127.87, 125.94, 125.41, 125.36, 122.90, 122.76, 118.22, 69.88, 42.76. MS (EI) *m/z* calculated for M^+ ($C_{14}H_{14}O$) = 198,1; found: 198 (2%), 179 (100%), 166, 157, 129; HPLC - Chiracel AD-H column was used and ee was found as 77%, (*i*-propanol:hexane 5:95, 1mL/min t_1 = 7.57 min ":S enantiomer", t_2 = 8.62 min "R enantiomer").

4.1.10. Preparation of R-(+)-1-(naphthalen-1-yl)-but-3-enyl acrylate (100)

A solution of 213 mg (1.07 mmol) of (**94**) in 3.0 mL of dichloromethane was cooled down to 0°C; then 175 mg (150 µL, 1.93 mmol) of acryloyl chloride (**58**) and 390 mg (540 µL, 3.85 mmol) of triethyl amine were sequentially added. The mixture was warmed to room temperature and stirred for 3 h under nitrogen atmosphere. The resulting mixture was filtered through a short pad of celite, poured into water, and the product was extracted with 3x25 mL of dichloromethane. The combined organic layer was concentrated under reduced pressure and purification of this residue by column chromatography on silica gel (ethyl acetate:hexane, 1:8) gave 186 mg of R-(+)-1-(naphthalen-1-yl)-but-3-enyl acrylate (**100**) with 69% yield. R_f = 0.63 (ethyl acetate:hexanes, 1:4); $[\alpha]_D^{26}$= +7.92° (c = 2.12, EtOAc); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.16 (d,1H, J=8.27 Hz), 7.88 (d,1H, J=8.27 Hz), 7.81 (d,1H, J=8.27 Hz), 7.61-7.44 (m,4H), 6.71-6.66 (m,1H), 6.47 (dd,1H, J=17.46 and 1.84 Hz), 6.21 (dd,1H, J=17.46 and 10.11 Hz), 5.89-5.74 (m,2H), 5.15-5.04 (m,2H), 2.87-2.81 (m,2H); ^{13}C-NMR (400MHz, $CDCl_3$) δ 165.59, 136.08, 134.04, 133.69, 131.21, 130.55, 129.15, 128.77, 128.73, 126.54, 125.89, 125.45, 124.11, 123.39, 118.23, 72.73, 40.60; Enantiomeric excess was found as 44% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 5:95, 1mL/min t_1 = 3.21 min "R enantiomer", t_2 = 4.22 min "S enantiomer").

4.1.11. Preparation of R-(+)-6-(naphthalen-1-yl)-5,6-dihydro-2H-pyran-2-one (82)

To a stirred solution of 73 mg (0.09 mmol) of Grubbs' catalyst (10 mol%) in 7.5 mL dichloromethane at 60 °C was added a solution of 180 mg (0.71 mmol) of **100** in 70 mL of dichloromethane. The resulting mixture was heated for 15 h. At the end of this period, the mixture was cooled to room temperature and concentrated under reduced

pressure. The crude was purified by column chromatography on silica gel (ethyl acetate:hexanes, 1:8) furnished 125 mg of R-(+)-6-(naphthalen-1-yl)-5,6-dihydro-2H-pyran-2-one (**82**) as a light yellow solid with 75% yield. R_f=0.14 (ethyl acetate:hexanes, 1:4). $[\alpha]_D^{23}$ = +172.88^o (c = 1.25, CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.00-7.82 (m,3H), 7.71 (d,1H, J=7.14 Hz), 7.58-7.45 (m,3H), 7.09-6.99 (m,1H), 6.25-6.17 (m,2H), 2.84-2.78 (m, 2H); ^{13}C-NMR (400MHz, $CDCl_3$) δ 164.54, 145.49, 134.07, 133.99, 130.16, 129.41, 128.38, 126.85, 126.07, 125.61, 124.39, 122.72, 121.89, 76.71, 31.38; Enantiomeric excess was found as 11% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 5:95, 1mL/min t_1 = 15.50 min, "R enantiomer", t_2 = 19.50 min "S enantiomer").

4.1.12. Preparation of R-1-(2-methylnaphthalen-1-yl)-but-3-en-1-ol (95)

In a 10 mL round-bottom flask equipped with a magnetic stirring bar and a condenser, 25 mg (14 µL, 0.13 mmol) of $TiCl_4$ was dissolved in 2.6 mL of dichloromethane. To this solution, 118 mg (120 µL, 0.41 mmol) of Ti(O*i*-Pr)$_4$ was added under nitrogen atmosphere. The resulting solution was allowed to warm up to room temperature for 1 h and then 61 mg (0.26 mmol) of silver (I) oxide was added. The mixture was stirred for 5 h without any exposure to direct day light. After dilution with 4.5 mL of dichloromethane, 150 mg (0.52 mmol) of (R)-BINOL (**59**) was added and stirred for 2 h. The resulting mixture was cooled down to -75 ^{o}C, and then treated sequentially with 446 mg (2.62 mmol) of 2-methyl-1-naphthaldehyde (**89**) and 953 mg (893 µL, 2.88 mmol) of allyltributyltin (**61**). The mixture was stirred for 18 h and allowed to warm up to room temperature. After the reaction was completed, the reaction mixture was filtered through a short pad of celite, then quenched with saturated $NaHCO_3$ solution, and extracted with 3x30 mL of ethyl acetate. The organic extracts were combined and dried over $MgSO_4$. After the removal of solvent under vacuum, purification of the crude by column chromatography on silica gel (ethyl acetate:hexanes, 1:8) furnished 104 mg of R-(+)-1-(2-methylnaphthalen-1-yl)-but-3-en-1-ol (**95**) as a colorless oil with 19% yield. R_f = 0.34 (ethyl acetate:hexanes, 1:6); $[\alpha]_D^{21}$= (Could not be measured) (c = 1.04, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.67 (d,1H, J=8.61 Hz), 7.81 (d,1H, J=7.82 Hz), 7.51-7.39 (m,2H), 7.29-7.24(m,1H),

5.96-5.84 (m,1H), 5.64-5.57(m,1H), 5.21 (ddd, 1H, J=17.21, 3.13 and 1.56 Hz), 5.17-5.12 (m,1H), 3.60-2.96 (m,1H), 2.76-2.67 (m,1H), 2.56 (s,3H), 2.20 (bs, 1H); ^{13}C-NMR (400MHz, $CDCl_3$) δ 135.30, 135.08, 133.38, 132.99, 131.26, 129.49, 128.70, 128.03, 125.63, 125.50, 124.64, 117.90, 71.54, 41.21, 21.00; Enantiomeric excess was found as 96% with HPLC - Chiracel AD-H column was used and ee was found as 93%, (*i*-propanol:hexane 5:95, 1mL/min t_1 = 3.70 min ":S enantiomer", t_2 = 4.50 min "R enantiomer").

4.1.13. Preparation of R-(+)-1-(2-methylnaphthalen-1-yl)-but-3-enyl acrylate (101)

A solution of 103 mg (0.49 mmol) of **95** in 3.0 mL of dichloromethane was cooled down to 0 ^{o}C; then 79 mg (71 μL, 0.88 mmol) of acryloyl chloride (**58**) and 118 mg (247 μL, 1.76 mmol) of triethyl amine were sequentially added. The mixture was warmed to room temperature and stirred for 3.5 h under nitrogen atmosphere. The resulting mixture was filtered through a short pad of celite, poured into water, and the product was extracted with 3x25 mL of dichloromethane. The combined organic layer was concentrated under reduced pressure and purification of this residue by column chromatography on silica gel (ethyl acetate:hexane, 1:10) gave 81 mg of R-(+)-1-(2-methylnaphthalen-1-yl)-but-3-enyl acrylate (**101**) as a light yellow oil with 62% yield. R_f = 0.47 (ethyl acetate:hexanes, 1:4); $[\alpha]_D^{18}$ = +15.15^{o} (c = 0.80, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.51 (d,1H, J=8.61 Hz), 7.82 (d,1H, J=7.82 Hz), 7.71 (d,1H, J=8.61 Hz), 7.54-7.48 (m,1H), 7.46-7.41 (m,1H), 7.29 (d,1H, J=8.22 Hz), 6.42 (dd,1H, J=17.22 and 1.56 Hz), 6.18 (dd,1H, J=17.22 and 10.56 Hz), 5.84-5.72 (m,2H), 5.14 (ddd,1H, J=17.22, 3.13, and 1.56 Hz), 5.10-5.05 (m,1H), 3.19-3.09 (m,1H), 2.92-2.83 (m,1H), 2.68 (s,3H); ^{13}C-NMR (400MHz, $CDCl_3$) δ 165.42, 134.28, 133.54, 133.27, 132.01, 131.08, 130.72, 129.34, 128.84, 128.53, 128.46, 125.73, 124.54, 117.92, 117.90, 72.83, 39.11, 20.92; Enantiomeric excess was found as 100% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 1:99, 0.7mL/min t_1 = 4.00 min. single peak).

4.1.14. Preparation of R-(+)-6-(2-methylnaphthalen-1-yl)-5,6-dihydro-2H-pyran-2-one (83)

To a stirred solution of 26 mg (0.03 mmol) of Grubbs' catalyst (10 mol%) in 3 mL dichloromethane at 60 ^{o}C was added a solution of 70 mg (0.27 mmol) of **101** in 26 mL of dichloromethane. The resulting mixture was heated for 14 h. At the end of this period, the mixture was cooled to room temperature and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (ethyl acetate:hexanes, 1:8) furnished 38 mg of R-(+)-6-(2-methylnaphthalen-1-yl)-5,6-dihydro-2H-pyran-2-one (**83**) as a light yellow oil with 60% yield. R_f=0.24 (ethyl acetate:hexanes, 1:2). $[\alpha]_D^{27}$ = + 136.79 (c = 0.31, CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.22 (d,1H, J=8.61 Hz), 7.82 (dd,1H, J=7.82, and 1.57 Hz), 7.75 (d,1H, J=8.22 Hz), 7.50-7.40 (m,2H), 7.29 (d,1H, J = 8.22 Hz), 7.06 (ddd,1H, J=9.78, 6.26, and 1.96 Hz), 6.30 (dd,1H, J=13.30 and 4.30 Hz), 6.23 (ddd,1H, J=9.78, 2.74, and 1.17 Hz), 3.27-3.15 (m,1H), 2.56 (s,3H), 2.55-2.44 (m,1H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 164.28, 145.70, 133.91, 133.59, 133.11, 131.03, 129.74, 129.35, 129.35, 129.32, 128.94, 126.20, 124.89, 124.46, 121.48, 76.58, 29.48, 20.84; Enantiomeric excess was found as 100% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 5:95, 1mL/min t_1 = 21.14 min, "single peak")

4.1.15. Preparation of R-(+)-1-(4-methylnaphthalen-1-yl)-but-3-en-1-ol (96)

In a 10 mL round-bottom flask equipped with a magnetic stirring bar and a condenser, 25 mg (14 μL, 0.13 mmol) of $TiCl_4$ was dissolved in 2.6 mL of dichloromethane. To this solution, 118 mg (120 μL, 0.41 mmol) of Ti(O*i*-Pr)$_4$ was added under nitrogen atmosphere. The resulting solution was allowed to warm up to room temperature for 1 h. and then 61 mg (0.26 mmol) of silver (I) oxide was added. The mixture was stirred for 5 h without any exposure to direct day light. After dilution with 4.5 mL of dichloromethane, 150 mg (0.52 mmol) of (R)-BINOL (**59**) was added and stirred for 2 h. The resulting mixture was cooled down to -75 ^{o}C, and then treated sequentially with 421 mg (2.47 mmol) of 4-methyl-1-naphthaldehyde (**90**) and 953 mg (893 μL, 2.88 mmol) of allyltributyltin (**61**). The mixture was stirred for 15.5 h and

allowed to warm up to room temperature. After the reaction was completed, the reaction mixture was filtered through a short pad of celite, then quenched with saturated $NaHCO_3$ solution, and extracted with 3x30 mL of ethyl acetate. The organic extracts were combined and dried over $MgSO_4$. After the removal of solvent under vacuum, purification of the crude by column chromatography on silica gel (ethyl acetate:hexanes, 1:10) furnished 71 mg of R-(+)-1-(4-methylnaphthalen-1-yl)-but-3-en-1-ol (**96**) as a yellow oil with 14% yield. R_f = 0.24 (ethyl acetate:hexanes, 1:6); $[\alpha]_D^{20}$= +57.72^o (c = 0.71, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.14-8.03 (m,2H), 7.58-7.51 (m,3H), 7.34 (d,1H, J=7.43 Hz), 6.00-5.89 (m,1H), 5.55-5.49(m,1H), 5.26-5.15 (m,2H), 2.81-2.73 (m,1H), 2.70 (s,3H), 2.66-2.57 (m,1H), 2.11 (bs,1H); ^{13}C-NMR (400MHz, $CDCl_3$) δ 137.51, 134.89, 134.04, 132.85, 130.37, 126.21, 125.67, 125.36, 124.98, 123.48, 122.55, 118.22, 69.96, 42.85, 19.58; Enantiomeric excess was found as 72% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 5:95, 1mL/min t_1 = 6.80 min ":R enantiomer", t_2 = 7.10 min "S enantiomer").

4.1.16. Preparation of R-(+)-1-(4-methylnaphthalen-1-yl)-but-3-enyl acrylate (102)

A solution of 70 mg (0.33 mmol) of **96** in 3.0 mL of dichloromethane was cooled down to 0 ^{o}C, then 55 mg (47 µL, 0.60 mmol) of acryloyl chloride (**58**) and 122 mg (167 µL, 1.21 mmol) of triethyl amine were sequentially added. The mixture was warmed to room temperature and stirred for 4 h under nitrogen atmosphere. The resulting mixture was filtered through a short pad of celite, poured into water, and the product was extracted with 3x25 mL of dichloromethane. The combined organic layer was concentrated under reduced pressure and purification of this residue by column chromatography on silica gel (ethyl acetate:hexane, 1:12) gave 50 mg of R-(+)-1-(4-methylnaphthalen-1-yl)-but-3-enyl acrylate (**102**) as a light yellow oil with 56% yield. R_f = 0.45 (ethyl acetate:hexanes, 1:8); $[\alpha]_D^{31}$ = +13.33^o (c = 2.73, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.21-8.16 (m,1H), 8.07-8.03 (m,1H), 7.60-7.52 (m,2H), 7.48 (d,1H, J=7.04 Hz), 7.32 (dd,1H, J=7.43 and 0.78 Hz), 6.70-6.65 (m,1H), 6.46 (dd,1H, J=17.22 and 1.56 Hz), 6.20 (dd,1H, J=17.22 and 10.56 Hz), 5.88-5.76 (m,2H), 5.13 (dd,1H, J=3.13 and 1.56 Hz), 5.09-5.04 (m,1H), 2.87-2.81 (m,2H), 2.69 (d,3H, J=0.78 Hz); ^{13}C-NMR (400MHz, $CDCl_3$) δ 165.36, 134.64, 133.96, 133.56, 132.84, 130.85,

130.39, 128.56, 126.02, 125.92, 125.49, 124.92, 123.63, 117.87, 72.51, 40.32, 19.58; Enantiomeric excess was found as 66% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 1:99, 1 mL/min t_1 = 2.80 min. "R enantiomer", t_2 = 3.16 min "S enantiomer").

4.1.17. Preparation of R-(+)-6-(4-methylnaphthalen-1-yl)-5,6-dihydro-2H-pyran-2-one (84)

To a stirred solution of 13 mg (0.02 mmol) of Grubbs' catalyst (10 mol%) in 1.5 mL dichloromethane at 60 ^oC was added a solution of 35 mg (0.13 mmol) of **102** in 14 mL of dichloromethane. The resulting mixture was heated for 14 h. At the end of this period, the mixture was cooled to room temperature and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (ethyl acetate:hexanes, 1:8) furnished 27 mg of R-(+)-6-(4-methylnaphthalen-1-yl)-5,6-dihydro-2H-pyran-2-one (**84**) as a light yellow solid with 87% yield. R_f=0.15 (ethyl acetate:hexanes, 1:4). $[\alpha]_D^{28}$ = + 114.64 (c = 0.21, CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.10-8.03 (m,1H), 8.01-7.95 (m,1H), 7.61-7.52 (m,3H), 7.35 (d,1H, J=7.04 Hz), 7.06-6.99 (m,1H), 6.24-6.15 (m,2H), 2.83-2.76 (m,2H), 2.71 (s,1H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 164.38, 145.27, 135.44, 132.83, 131.96, 130.03, 126.18, 126.10, 125.64, 125.14, 123.88, 123.01, 121.59, 76.79, 31.08, 19.61; Enantiomeric excess was found as 93% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 5:95, 1mL/min t_1 = 14.96 min, "R enantiomer", t_2 = 17.64 min "S enantiomer").

4.1.18. Preparation of R-(+)-1-(quinolin-4-yl)-but-3-en-1-ol (97)

In a 10 mL round-bottom flask equipped with a magnetic stirring bar and a condenser, 17 mg (10 μL, 0.1 mmol) of $TiCl_4$ was dissolved in 2.0 mL of dichloromethane. To this solution, 80 mg (80 μL, 0.28 mmol) of Ti(O*i*-Pr)$_4$ was added under nitrogen atmosphere. The resulting solution was allowed to warm up to room temperature for 1 h and then 40 mg (0.18 mmol) of silver (I) oxide was added. The mixture was stirred for 5 h without any exposure to direct day light. After dilution with 4.0 mL of dichloromethane, 100 mg (0.35 mmol) of (R)-BINOL (**59**) was added and stirred for 2 h. The resulting mixture was cooled down to -75 ^oC, and then treated

sequentially with 278 mg (1.77 mmol) of 4-quinolinecarboxyaldehyde (**91**) and 640 mg (598 μL, 1.93 mmol) of allyltributyltin (**61**). The mixture was stirred for 16 h and allowed to warm up to room temperature. After the reaction was completed, the reaction mixture was filtered through a short pad of celite, then quenched with saturated $NaHCO_3$ solution, and extracted with 3x30 mL of ethyl acetate. The organic extracts were combined and dried over $MgSO_4$. After the removal of solvent under vacuum, purification of the crude by column chromatography on silica gel (ethyl acetate:hexanes, 1:6) furnished 73 mg of R-(+)-1-(quinolin-4-yl)-but-3-en-1-ol (**97**) as a colorless oil with 21% yield. R_f = 0.22 (ethyl acetate:hexanes, 1:1); $[\alpha]_D^{21}$ = +67.08^o (c = 0.73, EtOH); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.71-8.65 (m,1H), 8.06-8.00 (m,1H), 7.97-7.91 (m,1H), 7.65-7.58 (m,1H), 7.55-7.45 (m,2H), 5.94-5.83 (m,1H), 5.52-5.46 (m,1H), 5.18-5.10 (m,2H), 4.30 (bs,1H), 2.74-2.65 (m,1H), 2.57-2.47 (m,1H); ^{13}C-NMR (400MHz, $CDCl_3$) δ 150.04, 149.94, 147.68, 133.94, 129.80, 128.98, 126.43, 125.31, 122.82, 118.50, 117.52, 68.78, 42.71; Enantiomeric excess was found as 65% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 5:95, 1mL/min t_1 = 14.70 min ":R enantiomer", t_2 = 17.09 min "S enantiomer").

4.1.19. Preparation of R-(-)-1-(quinolin-4-yl)-but-3-enyl acrylate (103)

A solution of 66 mg (0.30 mmol) of **97** in 3.0 mL of dichloromethane was cooled down to 0 ^{o}C, then 50 mg (43 μL, 0.55 mmol) of acryloyl chloride (**58**) and 110 mg (153 μL, 1.1 mmol) of triethyl amine were sequentially added. The mixture was warmed to room temperature and stirred for 3 h under nitrogen atmosphere. The resulting mixture was filtered through a short pad of celite, poured into water, and the product was extracted with 3x25 mL of dichloromethane. The combined organic layer was concentrated under reduced pressure and purification of this residue by column chromatography on silica gel (ethyl acetate:hexane, 1:8) gave 43 mg of R-(+)-1-(quinolin-4-yl)-but-3-enyl acrylate (**103**) as a yellow oil with 56% yield. R_f = 0.15 (ethyl acetate:hexanes, 1:4); $[\alpha]_D^{28}$= -5.50^o (c = 2.30, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.90 (d,1H, J=4.30 Hz) 8.18-8.07 (m,2H), 7.73 (ddd,1H, J=8.22, 6.65 and 1.17 Hz), 7.60 (ddd,1H, J=8.22, 7.04 and 1.17 Hz), 7.43 (d,1H, J=3.91 Hz), 6.62 (dd,1H, J=7.43 and 5.48 Hz), 6.49 (dd,1H, J=17.22 and 1.56 Hz), 6.22 (dd,1H, J=17.22 and 10.56 Hz), 5.90 (dd,1H, J=10.56 and 1.17 Hz), 5.83-5.71 (m,1H), 5.13-5.05 (m,2H),

2.85-2.71 (m,2H); ^{13}C-NMR (400MHz, $CDCl_3$) δ 165.08, 150.07, 148.39, 145.45, 132.41, 131.67, 130.46, 129.28, 128.01, 126.88, 125.19, 122.91, 118.74, 117.83, 71.21, 40.01; Enantiomeric excess was found as 100% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 1:99, 1 mL/min t_1 = 11.50 min. "single peak").

4.1.20. Preparation of R-(+)-6-(quinolin-4-yl)-5,6-dihydro-2H-pyran-2-one (85)

To a stirred solution of 12 mg (0.01 mmol) of Grubbs' catalyst (10 mol%) in 1.5 mL dichloromethane at 60 ^{o}C was added a solution of 30 mg (0.12 mmol) of **103** in 14 mL of dichloromethane. The resulting mixture was heated for 14 h. At the end of this period, the mixture was cooled to room temperature and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (ethyl acetate:hexanes, 1:8) furnished 9 mg of R-(+)-6-(quinolin-4-yl)-5,6-dihydro-2H-pyran-2-one (**85**) as a light yellow solid with 34% yield. R_f=0.14 (ethyl acetate:hexanes, 1:1). $[\alpha]_D^{26}$ = +313.91^{o} (c = 0.63, CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.91 (d,1H, J=4.70 Hz), 8.15 (dd,1H, J=8.61 and 0.78 Hz), 8.01 (dd,1H, J=8.61 and 0.78 Hz), 7.72 (ddd,1H, J=8.22, 6.65 and 1.17 Hz), 7.61-7.55 (m,2H), 5.96-5.85 (m,1H), 5.57-5.52 (m,1H), 5.27-5.21 (m,2H), 2.82-2.74 (m,1H), 2.58-2.49 (m,1H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 150.33, 149.20, 148.26, 133.73, 130.37, 129.06, 126.57, 125.35, 122.76, 119.21, 117.44, 68.85, 42.80, 29.67; Enantiomeric excess was found as 95% with HPLC -Chiracel AD-H column (*i*-propanol:hexane 5:95, 1mL/min t_1 = 6.42 min, "R enantiomer", t_2 = 7,15 min "S enantiomer").

4.1.21. Preparation of R-(+)-1-(quinolin-3-yl)-but-3-en-1-ol (98)

In a 10 mL round-bottom flask equipped with a magnetic stirring bar and a condenser, 25 mg (14 μL, 0.13 mmol) of $TiCl_4$ was dissolved in 2.6 mL of dichloromethane. To this solution, 118 mg (120 μL, 0.41 mmol) of Ti(O*i*-Pr)$_4$ was added under nitrogen atmosphere. The resulting solution was allowed to warm up to room temperature for 1 h and then 61 mg (0.26 mmol) of silver (I) oxide was added. The mixture was stirred for 5 h without any exposure to direct day light. After dilution with 4.5 mL of dichloromethane, 150 mg (0.52 mmol) of (R)-BINOL (**59**) was added

and stirred for 2 h. The resulting mixture was cooled down to -75 ^{o}C, and then treated sequentially with 412 mg (2.62 mmol) of 3-quinolinecarboxyaldehyde (**92**) and 953 mg (893 μL, 2.88 mmol) of allyltributyltin (**61**). The mixture was stirred for 16 h and allowed to warm up to room temperature. After the reaction was completed, the reaction mixture was filtered through a short pad of celite, then quenched with saturated $NaHCO_3$ solution, and extracted with 3x30 mL of ethyl acetate. The organic extracts were combined and dried over $MgSO_4$. After the removal of solvent under vacuum, purification of the crude by column chromatography on silica gel (ethyl acetate:hexanes, 1:6) furnished 253 mg of R-(+)-1-(quinolin-3-yl)-but-3-en-1-ol (**98**) as a yellow solid with 48% yield. R_f = 0.22 (ethyl acetate:hexanes, 1:1); $[\alpha]_D^{20}$ = +28.21^{o} (c = 2.53, EtOH); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.72 (s,1H), 8.06 (s,1H), 8.00 (d,1H, J=8.22 Hz), 7.71 (d,1H, J=8.22 Hz), 7.65-7.58 (m,1H), 7.51-7.44 (m,1H), 5.85-5.72 (m,1H), 5.14-5.06 (m,2H), 4.93-4.87 (m,1H), 4.15 (bs,1H), 2.59-2.52 (m,2H); ^{13}C-NMR (400MHz, $CDCl_3$) δ 149.19, 147.15, 136.83, 133.66, 132.78, 129.20, 128.66, 127.71, 127.69, 126.70, 118.71, 71.06, 43.56; Enantiomeric excess was found as 93% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 5:95, 1mL/min t_1 = 23.90 min "S enantiomer", t_2 = 26.40 min "R enantiomer").

4.1.22. Preparation of R-(+)-1-(quinolin-3-yl)-but-3-enyl acrylate (104)

A solution of 248 mg (1.25 mmol) of **98** in 3.0 mL of dichloromethane was cooled down to 0 ^{o}C, then 205 mg (183 μL, 2.26 mmol) of acryloyl chloride (**58**) and 456 mg (634 μL, 4.51 mmol) of triethyl amine were sequentially added. The mixture was warmed to room temperature and stirred for 4 h under nitrogen atmosphere. The resulting mixture was filtered through a short pad of celite, poured into water, and the product was extracted with 3x25 mL of dichloromethane. The combined organic layer was concentrated under reduced pressure and purification of this residue by column chromatography on silica gel (ethyl acetate:hexane, 1:8) gave 171 mg of (R)-(+)-1-(quinolin-3-yl)-but-3-enyl acrylate (**104**) as a yellow solid with 54% yield. R_f = 0.26 (ethyl acetate:hexanes, 1:4); $[\alpha]_D^{18}$ = +74.37^{o} (c = 1.71, EtOH); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.92 (d,1H, J=2.35 Hz) 8.11-8.06 (m,2H), 7.79 (d,1H, J=8.22 Hz) 7.67 (ddd,1H, J=8.22, 6.65 and 1.56 Hz), 7.55-7.48 (m,1H), 6.43 (dd,1H, J=17.61 and 1.56 Hz), 6.20-6.11 (m,1H), 6.09-6.04 (m,1H), 5.53 (dd,1H, J=10.56 and 1.56 Hz), 5.78-

5.66 (m,1H), 5.12-5.04 (m,2H), 2.86-2.77 (m,1H), 2.75-2.66 (m,1H); ^{13}C-NMR (400MHz, $CDCl_3$) δ 165.08, 149.21, 147.74, 133.69, 132.48, 132.18, 131.35, 129.53, 129.13, 128.04, 127.77, 127.42, 126.82, 118.89, 73.34, 40.26; Enantiomeric excess was found as 92% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 1:99, 1 mL/min t_1 = 19.50 min. "R enantiomer", t_2 = 21.50 min "S enantiomer").

4.1.23. Preparation of R-(+)-6-(quinolin-3-yl)-5,6-dihydro-2H-pyran-2-one (86)

To a stirred solution of 57 mg (0.07 mmol) of Grubbs' catalyst (10 mol%) in 5 mL dichloromethane at 60 ^{o}C was added a solution of 146 mg (0.58 mmol) of **104** in 58 mL of dichloromethane. The resulting mixture was heated for 13.5 h. At the end of this period, the mixture was cooled to room temperature and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (ethyl acetate:hexanes, 1:6) furnished 80 mg of R-(+)-6-(quinolin-3-yl)-5,6-dihydro-2H-pyran-2-one (**86**) as a yellow solid with 62% yield. R_f=0.11 (ethyl acetate:hexanes, 1:2). $[\alpha]_D^{22}$= +205.76 (c = 0.78, EtOH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.92 (d,1H, J=1.96 Hz), 8.26 (s,1H), 8.12 (d,1H, J=8.22 Hz), 7.85 (d,1H, J=8.22 Hz), 7.78-7.72 (m,1H), 7.62-7.55 (m,1H), 7.06-6.99 (m,1H), 6.20 (dd,1H, J=9.78 and 1.56 Hz), 5.73-5.66 (m,1H), 2.79-2.73 (m,2H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 163.48, 148.26, 148.06, 144.56, 133.30, 131.18, 130.04, 129.28, 127.98, 127.45, 127.26 121.83, 77.11, 31.52; Enantiomeric excess was found as 97% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 5:95, 1mL/min t_1 = 27.46 min, "S enantiomer", t_2 = 33.19 min "R enantiomer").

4.1.24. Preparation of R-(+)-1-(3-phenoxyphenyl)-but-3-en-1-ol (99)

In a 10 mL round-bottom flask equipped with a magnetic stirring bar and a condenser, 25 mg (14 µL, 0.13 mmol) of $TiCl_4$ was dissolved in 2.6 mL of dichloromethane. To this solution, 118 mg (120 µL, 0.41 mmol) of Ti(O*i*-Pr)$_4$ was added under nitrogen atmosphere. The resulting solution was allowed to warm up to room temperature for 1 h and then 61 mg (0.26 mmol) of silver (I) oxide was added. The mixture was stirred for 5 h without any exposure to direct day light. After dilution

with 4.5 mL of dichloromethane, 150 mg (0.52 mmol) of (R)-BINOL (**59**) was added and stirred for 2 h. The resulting mixture was cooled down to -75 ^{o}C, and then treated sequentially with 519 mg (2.62 mmol) of 3-phenoxybenzaldehyde (**93**) and 953 mg (893 µL, 2.88 mmol) of allyltributyltin (**61**). The mixture was stirred for 40 h and allowed to warm up to room temperature. After the reaction was completed, the reaction mixture was filtered through a short pad of celite, then quenched with saturated $NaHCO_3$ solution, and extracted with 3x30 mL of ethyl acetate. The organic extracts were combined and dried over $MgSO_4$. After the removal of solvent under vacuum, purification of the crude by column chromatography on silica gel (ethyl acetate:hexanes, 1:14) furnished 72 mg of R-(+)-1-(3-phenoxyphenyl)-but-3-en-1-ol (**99**) as a colorless oil with 11% yield. R_f = 0.12 (ethyl acetate:hexanes, 1:10); $[\alpha]_D^{22}$ = +31.66^{o} (c = 0.72, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.38-7.28 (m,3H), 7.17-7.08 (m,2H), 7.06-7.00(m,3H), 6.92 (ddd,1H, J=8.22, 2.74 and 1.17 Hz), 5.86-5.74 (m,1H), 5.19-5.15 (m,1H), 4.74-4.68 (m,1H), 2.43-2.57 (m,2H), 2.23 (bd,1H, J=2.74 Hz); ^{13}C-NMR (400MHz, $CDCl_3$) δ 157.33, 157.12, 146.00, 134.15, 129.72, 129.70, 123.23, 120.58, 118.84, 117.83, 116.30, 72.87, 43.79; Enantiomeric excess was found as 76% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 5:95, 1mL/min t_1 = 6.90 min "R enantiomer", t_2 = 7.80 min "S enantiomer").

4.1.25. Preparation of R-(+)-1-(3-phenoxyphenyl)-but-3-enyl acrylate (105)

A solution of 66 mg (0.28 mmol) of **99** in 3.0 mL of dichloromethane was cooled down to 0 ^{o}C, then 45 mg (39 µL, 0.49 mmol) of acryloyl chloride (**58**) and 100 mg (139 µL, 0.99 mmol) of triethyl amine were sequentially added. The mixture was warmed to room temperature and stirred for 3.5 h under nitrogen atmosphere. The resulting mixture was filtered through a short pad of celite, poured into water, and the product was extracted with 3x25 mL of dichloromethane. The combined organic layer was concentrated under reduced pressure and purification of this residue by column chromatography on silica gel (ethyl acetate:hexane, 1:12) gave 61 mg of R-(+)-1-(3-phenoxyphenyl)-but-3-enyl acrylate (**105**) as a light yellow oil with 76% yield. R_f = 0.50 (ethyl acetate:hexanes, 1:8); $[\alpha]_D^{21}$= +28.67^{o} (c = 0.43, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.38-7.28 (m,3H), 7.15-7.08 (m,2H), 7.06-7.00 (m,3H), 6.92

(ddd,1H, J=8.22, 2.35 and 0.78 Hz), 6.43 (dd,1H, J=17.22 and 1.56 Hz), 6.16 (dd,1H, J=17.22 and 10.17 Hz),5.90-5.82 (m,2H), 5.79-5.67 (m,1H), 5.13-5.05 (m,2H), 2.73-2.55 (m,2H); ^{13}C-NMR (400MHz, $CDCl_3$) δ 165.21, 157.27, 156.94, 142.04, 132.92, 130.94, 129.71, 129.70, 128.41, 123.29, 121.22, 118.86, 118.23, 118.06, 116.80, 74.83, 40.72; Enantiomeric excess was found as 75% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 1:99, 1 mL/min t_1 = 4.50 min. "S enantiomer", t_2 = 4.80 min "R enantiomer").

4.1.26. Preparation of R-(+)-6-(3-phenoxyphenyl)-5,6-dihydro-2H-pyran-2-one (87)

To a stirred solution of 15 mg (0.02 mmol) of Grubbs' catalyst (10 mol%) in 2 mL dichloromethane at 60 ^{o}C was added a solution of 43 mg (0.15 mmol) of **105** in 17 mL of dichloromethane. The resulting mixture was heated for 14.5 h. At the end of this period, the mixture was cooled to room temperature and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (ethyl acetate:hexanes, 1:8) furnished 35 mg of R-(+)-6-(3-phenoxyphenyl)-5,6-dihydro-2H-pyran-2-one (**87**) as a yellow solid with 91% yield. R_f=0.30 (ethyl acetate:hexanes, 1:2). $[\alpha]_D^{29}$ = + 115.0 (c = 0.34, CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39-7.30 (m,3H), 7.17-6.92 (m,7H), 6.12 (ddd,1H, J=9.78, 2.35 and 1.56 Hz), 5.45-5.37 (m,1H), 2.70-2.55 (m,2H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 163.80, 157.57, 156.75, 144.74, 140.39, 130.03, 129.80, 123.53, 121.61, 120.69, 118.99, 118.74, 116.40, 78.77, 31.52; Enantiomeric excess was found as 77% with HPLC - Chiracel AD-H column (*i*-propanol:hexane 5:95, 1 mL/min t_1 =14.99 min, "S enantiomer", t_2 = 16.82 min "R enantiomer").

4.2. Biological Part (Measuring Cell Viability (MTT Tests))

4.2.1. General Methods

Human Prostat Cancer (PC-3) cell line was kindly provided by Associate Professor Kemal Sami Korkmaz (Ege University, Engineering Faculty, Department of

Bioengineering), human brest cancer (MCF-7) cell line was obtained from Şap Institude. PC3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum (FBS), 100µg/ml streptomycin/100IU/ml penicillin, MCF7 cell line was maintained in Roswell Park Memorial Institude-1640 (RPMI-1640) containing 15% FBS (BIO-IND), 100µg/ml streptomycin/100IU/ml penicillin incubated at 37 ºC in the dark with 5% CO_2 humidified incubator and passaged when they reached 80-85% confluency. Cells used in experiments were maintained from 10-20th passages.

4.2.2. MTT Test for Compounds 100-105 and 82-87

To investigate the cytotoxic activity of the compounds, 95µl of cell suspension was inoculated into 96-well microculture plates at $1x10^4$ cells dencity per well in culture media containing FBS, penicillin/streptomycin. Compounds were dissolved in dimethyl sulfoxide (DMSO) (Sigma Chemical Co.), filter sterilized, diluted at the appropriate concentrations with the cultura medium. In all well, 1% DMSO concentration was fixed. Dilutions of compounds were freshly prepared before each experiments. After 24h cultivation for cell attachment, extracts were added at final concentration 50, 25, 1, 0.5, 0.1, 0.05, and 0.01 µM for triplicate assay. Cells were treated with the extracts for 48 hours and cytotoxic effects were determined by tetrazolium (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma Chemical Co.) based colorimetric assay. This method depends on the cleavage of tetrazolium salt to purple formazan crystals by mitochondrial enzymes of metabolically active cells (Ciapetti, et al. 1993). Briefly; 4 hours before the end of incubation period, medium of the cells was removed and wells were washed by pre-warmed phosphate-buffered saline (PBS) to remove any trace of compounds and to prevent colour interferance while optical density determination. MTT stock solution (5mg/ml) was diluted at 1:10 ratio into complete culture media, 100µl of MTT dilution was added into each well and incubated. After 3.5 hours plates were centrifuged at 1800 rpm for 10 minute at room temperatures to avoid accidental removal of formazan crystals. Crystals were dissolved with 100µl DMSO. The absorbance was determined at 540nm. Results were represented as a percentage viability and calculated by the following formula (Equation 4.1):

$$\% \text{ viability}=[(ODs-OD_B/ODc-OD_B)x100] \quad (4.1)$$

OD_B indicated the optical density of blank, ODs indicated the optical density of sample and ODc indicated the optical density of control.

4.2.3. MTT Tests for Compounds 75-76.

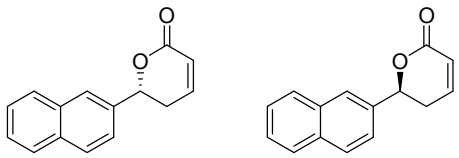
Four cell lines including PC-3, DU145, LNCaP, and MCF-7 were obtained from the ATCC (USA) culture collection. Cells were cultured in RPMI-1640 (Invitrogen, USA) or DMEM (Invitrogen, USA) supplemented with 5-10% fetal bovine serum (Sigma, USA), by additions of 100 IU/mL penicillin and l μg/mL streptomycin. Cells were grown in humidified atmosphere with 5% CO_2 at 37 ^oC. Cytotoxic effects of compounds were analyzed by MTT assay which is based on the cellular reduction of the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT, Sigma Chemicals) to a blue formazan product by mitochondrial dehydrogenases of viable cells. Cell proliferation was determined by adding 0.5 μg/mL per well, prepared as a sterile stock-solution of 5 mg/mL in Dulbeccos-phosphate buffered saline (Gibco), diluted 1:10 in medium prior to use. Medium was removed 4 h later and blue formazan crystals solubilized in 200 μL 100% dimethylsulfoxate (DMSO) per well. Amounts of blue formazan product were quantified at 570-690 nm using a microplate reader (Versamax, Tunable Microplate Reader, USA). For all cell lines, strong correlations between numbers of cells present and amounts of MTT formazan product were observed. Each cell type was incubated with various doses and for 72 h at 37 ^oC and subjected to MTT assays to measure IC_{50} values. The data were obtained from three independent assays using two wells for each assay. Cell viability was calculated as % cell viability.

CHAPTER 5

RESULTS AND DISCUSSION

The mechanism of action of the goniothalamin derivatives is not fully understood yet. Several studies have been done to explain the mechanism of cytotoxicity of goniothalamin. In these studies it was demonstrated that **23** is a potential genotoxic substance (Tsafe, et al. 2004). Beside the caspase-9 activation and loss of mithochondrial membrane potential of the HL-60 luekemia cells (Inayat-Hussain, et al. 2003), it is proposed that **23** is responsible for an increase in Bax (pro-apoptotic protein) levels (Teoh and Azimahtol 1999) (Chien and Pihie 2003) and for the activation of p53 tumor supressor protein (Menakshii, et al. 2000). In molecular level, it was proposed that the Michael acceptor in the pyran-2-one, possibly accepts the nucleophilic attack from the amino acid residues of proteins, is essential for anti-tumor activity (Fatima, et al. 2006).

In literature, there are more than 20 mechanistic explanation for the drug-enzyme interactions. In these proposals, conformational changes in drug and enzyme play important roles for activity. In drug design, often rotation around sigma bonds in the drug candidate can be restricted by preparing the conformationally constrained analogues to find the best conformational structure for the maximal inhibition of enzymes. Goniothalamin has only two sigma bonds which can rotate freely in the linker part. It is possible to minimize the conformational changes in (R)- and (S)-goniothalamin by replacing the styrene part with a naphthalene substituent to form conformationally constrained analogues **75** and **76** (Figure 5.1).



75 76

Figure 5.1. Proposed conformationally constrained goniothalamin analogues **75**, **76**.

Minimized energy conformation of (R)-goniothalamin (**23**), and conformationally constrained analogue **75** are shown in Figure 5.2 and 5.3 respectively. Main difference in these two structure is non-planar styrene structure in **23** was replaced with a fully planar naphthyl unit in **75**. As discussed before, it is proposed that the Michael acceptor in the lactone ring accepts the nucleophilic attack from possible target enzyme. If it is so, at the begining goniothalamin should fit the active site. Conformational changes in the styrene part may effect the kinetics of enzyme-drug complex formation. On the other hand, compounds **75** and **76** have less possible conformational changes which may increase the rate of the possible formation of enzyme-drug complex formation, which may improve the cytotoxicity of the lead compound **23**.

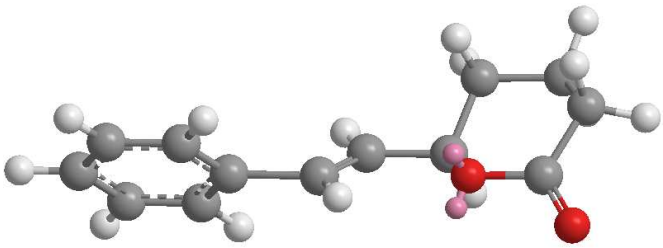


Figure 5.2. Minimized energy conformation of (R)-goniothalamin (**23**). Calculation was performed by using CHEM 3D Ultra software, equipped with modified version of Allinger's MM2 force field.

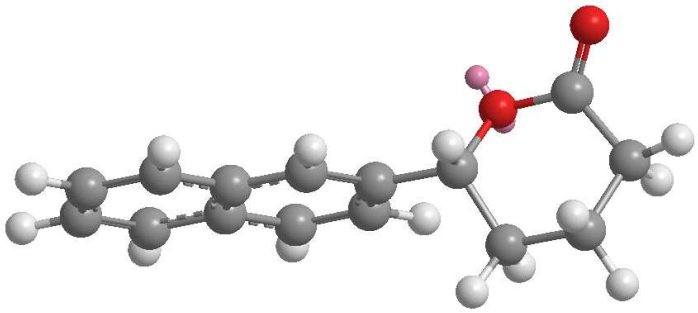
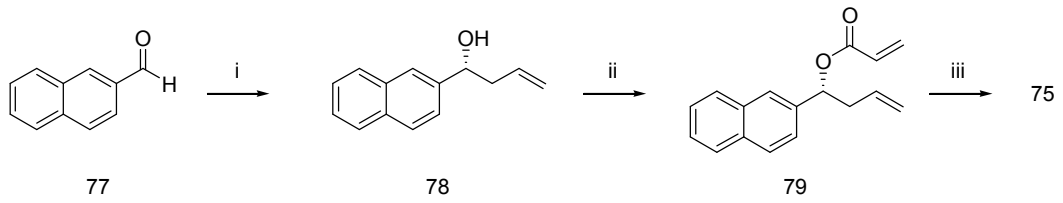


Figure 5.3. Minimized energy conformation of **75**. Calculation was performed by using CHEM 3D Ultra software, equipped with modified version of Allinger's MM2 force field.

5.1. Asymmetric Synthesis of Conformationally Constrained (R)- and (S)-Goniothalamin Analogues (75, and 76)

Asymmetric synthesis of the conformationally constrained analogues **75** and **76** were done via the route described by Fatima, et al. 2005. As shown in Figure 5.4, 2-naphthaldehyde (**77**) was used as starting material, which was selectively converted to (R)-homoallylic alcohol (**78**) through the asymmetric induction of allyl group in the presence of catalyst **60** with 59% yield and 83% ee. Enantiomeric excess of compound **78** was determined by performing HPLC study with Chiracel AD-H Column, (*i*-propanol:hexane 10:90, 1mL/min t_1 = 5.51 min "major enantiomer", t_2 = 5.97 min "minor enantiomer"). Calculated enantiomeric excess by optical rotation was also in agreement with literature value (Teo, et al. 2005). Obtained alcohol **78** was then treated with acryloyl chloride in the presence of triethyl amine to form ester **79** with 53% yield and 79% ee. Final step was the ring closing metathesis with Grubbs' catalyst of **79** to form **75** with 88% yield and 76% ee, HPLC study was performed also for **75** (*i*-propanol:hexane 5:95, 1mL/min t_1 = 17.66 min "major enantiomer", t_2 = 18.27 min "minor enantiomer").

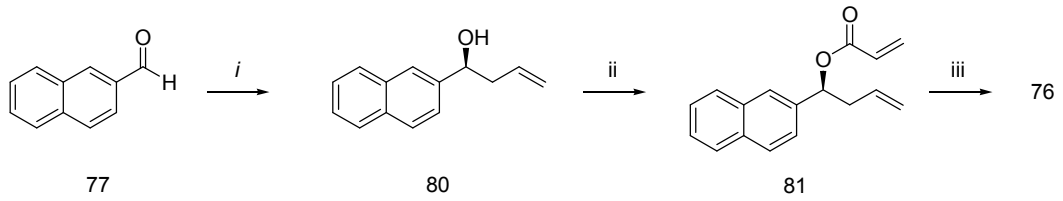


Conditions: i (R)-BINOL (10 mol%), $Ti(Oi\text{-}Pr)_4$ (15 mol%), $TiCl_4$ (5 mol%), allyltributyltin, -20 ^oC, 24 h (59%; 83% ee). ii acryloyl chloride, Et_3N, CH_2Cl_2, 0 ^oC (53%; 79%ee); iii Grubbs' catalyst, 60 ^oC, CH_2Cl_2 (88%; 76%ee).

Figure 5.4. Asymmetric synthesis of conformationally constrained analogues of (R)-Goniothalamin (**75**).

Same procedure was followed for the synthesis of compound **76**, only difference was the usage of (S)-BINOL to prepare catalyst to form allylic alcohol (**80**) and alcohol was obtained with 25% yield and 81% ee. Enantiomeric excess of the compound was determined by performing HPLC study with Chiracel AD-H Column, for alcohol **80**

(*i*-propanol:hexane 10:90, 1mL/min t_1 = 5.51 min "major enantiomer", t_2 = 5.97 min "minor enantiomer"). Then alcohol **80** was treated with acryloyl chloride in the presence of triethyl amine to form ester **81** with 73% yield and 81% ee. Chiracel AD-H Column HPLC study was performed for **81** (*i*-propanol:hexane 5:95, 1mL/min t_1 = 3.21 min "minor enantiomer", t_2 = 4.23 min "major enantiomer"). Final step was again the ring closing metathesis with Grubbs' catalyst, ester was lactonized with 73% yield and 43% ee, HPLC study was performed for the compound **76** in Chiracel AD-H Column (*i*-propanol:hexane 5:95, 1mL/min t_1 = 17.51 min "minor enantiomer", t_2 = 18.25 min "major enantiomer").



Conditions: i (S)-BINOL (10 mol%), $Ti(Oi\text{-}Pr)_4$ (15 mol%), $TiCl_4$ (5 mol%), allyltributyltin, -20 ^oC, 24 h (26%; 81% ee). ii acryloyl chloride, Et_3N, CH_2Cl_2, 0 ^oC (73%; 81%ee); iii Grubbs' catalyst, 60 ^oC, CH_2Cl_2 (73%; 43%ee).

Figure 5.5. Asymmetric synthesis of conformationally constrained analogues of (S)-goniothalamin (**76**).

5.2. Anti-Tumor Properties of Conformationally Constrained Analogues of (R)- and (S)-Goniothalamin (75, and 76)

Anti-tumor properties of compounds **75** and **76** were evaluated against DU-145, LNCAP, MCF-7, and PC-3 cancer cell line in vitro by using routine MTT tests. To compare the cytotoxicities of **75** and **76**, R-goniothalamin **23** was also tested against same cancer cell lines at 50, 25, 13, and 6.2 μM concentrations. As positive control in MTT test, camptothecin and etoposide were applied at 29, and 20 μM concentrations respectively. Results of the MTT tests were summerized in Figure 5.6.

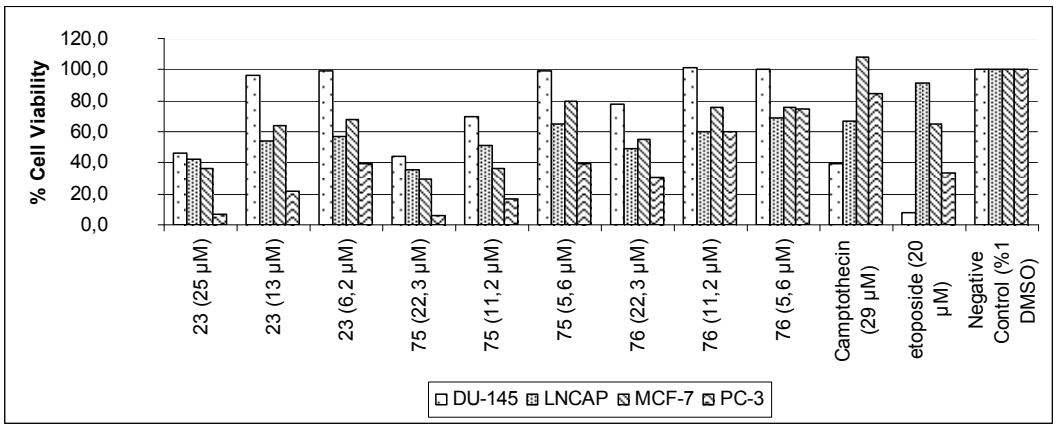


Figure 5.6. Relative cytotoxicities of the analogues compounds **23, 75** and **76** against DU-145, LNCAP, MCF-7, PC-3 cancer cell lines. Camptothecin and etoposide were used as positive control. (Data provided by Assoc. Prof. Dr. Kemal Korkmaz.)

All of the tested compounds inhibits the proliferation of all cancer cell lines more than 95% at 50 µM concentrations. Cytotoxicities of the compounds against DU-145 cell lines from most potent to lowest can be as etoposide > **23**≈**75**≈ camptothecin > **76**. When the tested cancer cell line was LNCAP, R-goniothalamin analogue **75** is the most potent which is slightly better than **23**. On the other hand S-goniothalamin analogue **76** is slightly less cytotoxic compare to **23**. Same results were observed when the compounds tested against MCF-7 and PC-3 cell lines. Concentration dependent % cell viability results were used to calculate the IC_{50} values for **23**, **75** and **76** (Table 5.1.).

Table 5.1. IC_{50} (µM) values for **23**, **75**, and **76**.[a]

Compound	DU-145	LNCAP	MCF-7	PC-3
23	28	12	19	4
75	19	11	12	3
76	37	15	28	12

[a] Concentration (average of three experiments) that is needed to inhibit 50% of the cell growth. Values are determined by using GraphPad Prism software (nonlinear regression analysis, r^2 > 0,9). (Data provided by Assoc. Prof. Dr. Kemal Korkmaz.)

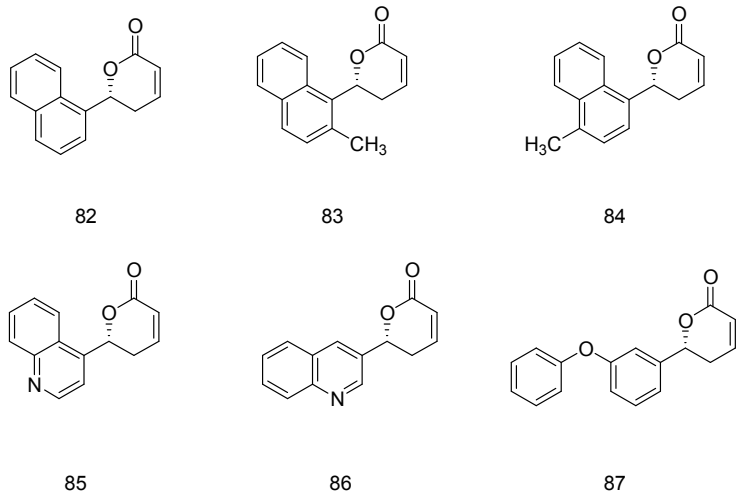
As it can be seen from table, conformationally constrained R-goniothalamin analogue **75** has slightly more antiproliferative property than R-goniothalamin (**23**). Meanwhile, conformationally constrained S-goniothalamin analogue **76** has less proliferative property than **75** (almost half of it) and **23**.

These preliminary results concluded that restriction of rotation around sigma bond in the styrene sub-unit enhances the cytotoxic activity slightly, and (S)-conformationally constrained analogue **76** is less active than its R enantiomer **75** which is in agreement with literature. (Fatima, et al. 2006)

5.3. Asymmetric Synthesis of Further Conformationally Restricted Naphthyl and Quinoylypyranones

Preparation of conformationally constrained analogues of a drug canditate is a useful approach, unless too much steric factors added to the lead compounds. Compounds **75** and **76** has an additional -HC=CH- steric factor as compared to the lead compound **23**. The source of the slight increase in cytotoxic activity can either be due to the restriction of rotation around sigma bond or the presence of the additional atoms which may play steric role. At this point, it is necessary to design new derivatives to clarify the questions.

In this regard, synthesis of six new 6-aryl substituted 5,6-dihydro-2H-pyran-2-ones **82**-**87** were planned. Structure of those were shown in Figure 5.7.



82 83 84

85 86 87

Figure 5.7. Structure of proposed 6-aryl substituted 5,6-dihydro-2H-pyran-2-ones **82**-**87**.

If the reason of slightly enhanced cytotoxic activity of **75** is steric it may be helpful to synthesize these new compounds to further enhance the activity. As discussed before, main pharmocophore of the lead compound was the Michael acceptor part of ring C in compound **75**. Ring A is relatively away from ring C, and rotation around the sigma bond may not create strong steric interactions as much as compound **88** does. (Figure 5.8)

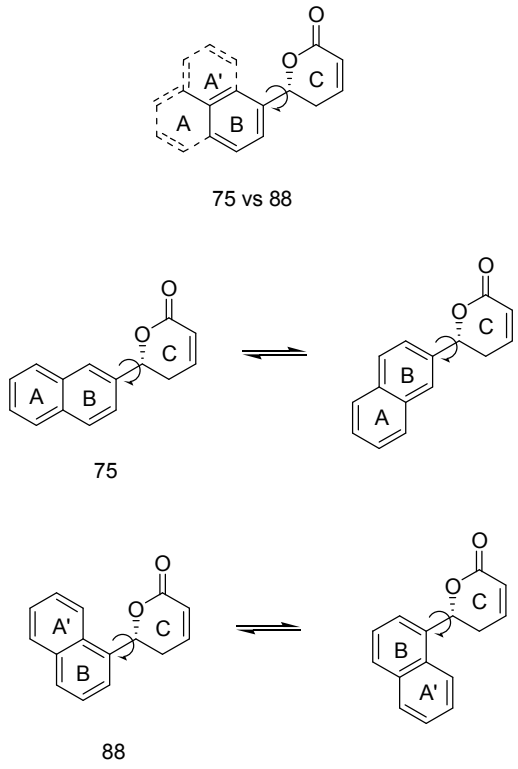


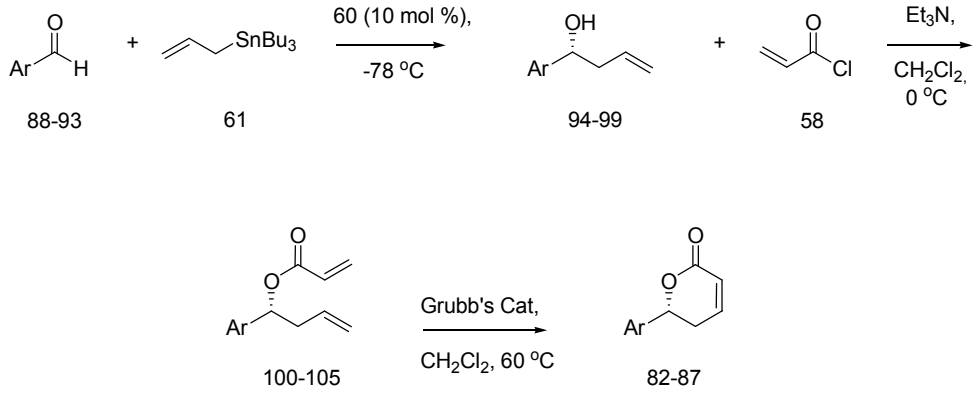
Figure 5.8. Comparison of the conformationally restricted analogues **75-88**.

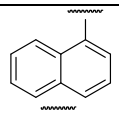
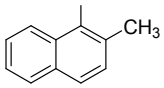
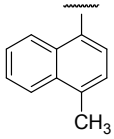
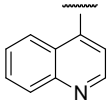
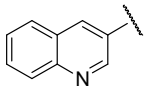
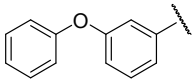
Compound **86** has the similar steric size with **75** and **76** which may help to understand the steric effect on activity of the compound. Only unexpected interference may be possible hydrogen bonding of nitrogen atom in the quinoline structure. Another alternative may be compound **87** which has crowded aryl ring attached to 2-pyranone ring.

The synthesis of compounds **82-87** were performed according to the same route used for the synthesis of compounds **23** and **75**. Yields and ee% of the reactions were given in Table 5.2. Catalytic asymmetric allylation of chosen aldehydes **88-93** gave the corresponding alcohols **94-99** in low yields with moderate enantioselection (65-93% ee). Although this method is reported in high yields and enantioselection, we could not

achieved smilar success from this methodology, which might be due to the possible moisture left in the solvent or reaction vessel. Enantiomeric excess of the resulting alcohols were calculated by applying HPLC studies with Chiracel AD-H Column.

Table 5.2. Synthesis of the 5-substituted α,β-unsaturated δ-lactones derivatives **82-87**.



	Ar	Product (% yield, %ee)	Product (% yield, %ee)	Product (% yield, %ee)
88		**94** (41, 77)	**100** (69, 44)	**82** (75, 11)
89		**95** (19, 97)	**101** (62, 100)	**83** (60, 100)
90		**96** (14, 72)	**102** (56, 66)	**84** (87, 93)
91		**97** (21, 65)	**103** (56, 100)	**85** (34, 95)
92		**98** (48, 93)	**104** (54, 92)	**86** (62, 97)
93		**99** (11, 76)	**105** (76, 75)	**87** (91, 77)

Alcohols **94-99** were successfully converted to their acryloyl ester by treating them with acryloyl chloride in the presence of triethyl amine with 54-76% yield. In the last steps, formed acryloyl esters **100-105** were transformed to pyranone derivatives **88-93** with 34-91% yields.

Although the followed synthetic route for the synthesis of 5-aryl substituted-5,6-dihydro-2H-2-pyranone system was found useful, the first step was a little bit problematic in our hands.

5.4. Surprising Cytotoxic Properties of the Isolated Acrylate Intermediates

Structure activity relationship (SAR) studies of goniothalamin implies that the Michael acceptor may be critical for the cytotoxicity. But it never be questioned whether the cyclic structure is required or not. When we looked at compounds **100-105**, all of these compounds have a Michael acceptor in their structure. Although the ring structure has been replaced by an acyclic allylalcohol acrylate, those may also found to be cytotoxic. In this cause, cytotoxicity of these compounds were tested against MCF-7 and PC-3 cell lines. Standard MTT test were employed and the results have ben shown in Figure 5.9, 5.10, 5.11, and 5.12. Compounds have been tested in seven different concentration. PC-3 test results have been shown in Figure 5.9 for above 1μM concentrations and in Figure 5.10 for concentrations below 1 μM respectively.

As shown in Figure 5.9 and 5.10 for PC-3 cell line most, active acrylate esters are **102** and **103**. Below 1μM concentrations, all of the compounds has very limited anti-proliferative activity. Although acrylate esters **101-103** shows comparable amount of cytotoxicity as goniothalamin does, there is no enhancement any of tested acrylate compounds.

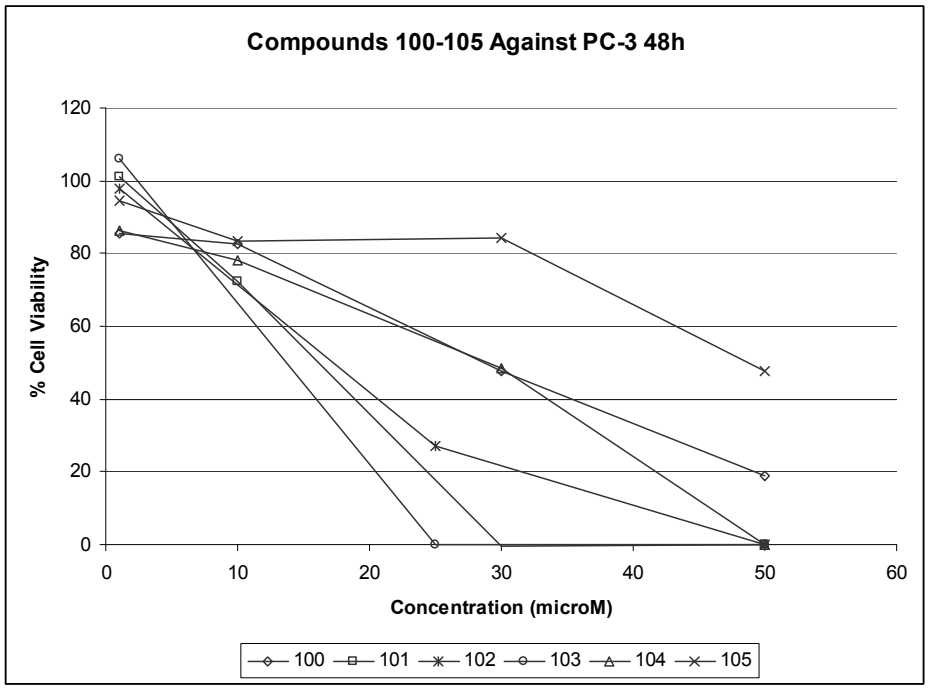


Figure 5.9. Concentration dependent cytotoxicities of compounds **100-105** against PC-3 cell lines for concentrations above 1μM.

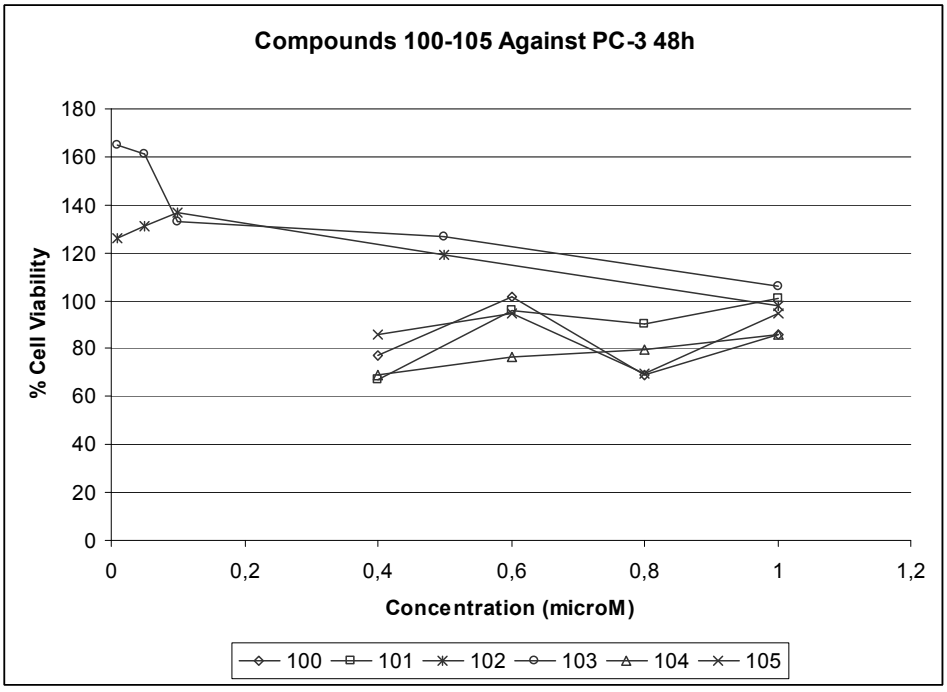


Figure 5.10. Concentration dependent cytotoxicities of compounds **100-105** against PC-3 cell lines for concentrations below 1μM.

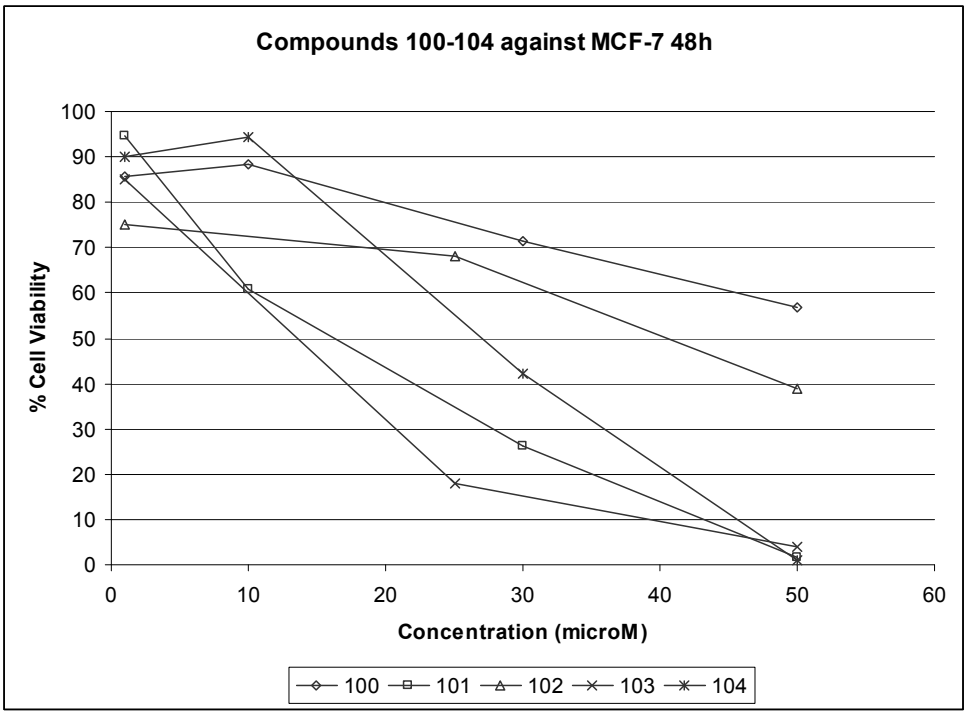


Figure 5.11. Concentration dependent cytotoxicities of compounds **100-105** against MCF-7 cell lines for concentrations above 1μM.

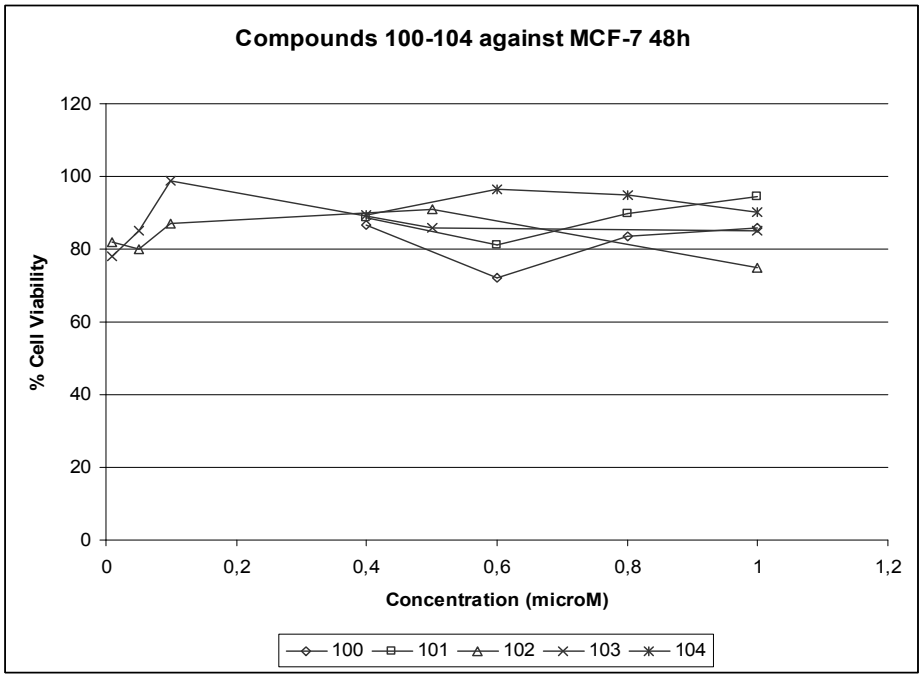


Figure 5.12. Concentration dependent cytotoxicities of compounds **100-105** against MCF-7 cell lines for concentrations below 1μM.

For MCF-7 cell line, the case is similar, and compounds **101**, **103** and **104** are the most active. IC_{50} values for the tested compounds **100-105** were calculated and shown in Table 3. Similar cytotoxicity trend has been observed from the table.

Table 5.3. IC_{50} concentrations for compounds **100-105** against PC-3 and MCF-7 cell lines.[a]

	MCF-7	**PC-3**
100	>50	22.9
101	10.4	8.8
102	33.1	45
103	13.2	15
104	28.8	16.3
105	>50	>50

[a] Concentration that inhibit cell proliferation 50% (average of the three replicate). Numbers are calculated in GraphPad Prism program in μM. (nonlinear regression analysis, $r^2 > 0,9$) (Measurements carried out by Specialist Özgür Yılmazer.)

5.5. Anti-Tumor Properties of Further Conformationally Restricted Naphthyl and Quinoylypyranones

Similar to acryloyl esters **100-105**, the conformationally constrained analogues **82-87** were also tested against the same cancer cell lines (PC-3 and MCF-7) in seven different concentration by using standard MTT test. Results were shown in Figure 5.13, 5.14, 5.15, and 5.16. test results for PC-3 cell lines have been shown in Figure 5.13 for concentrations above 1μM and in Figure 5.14 for concentrations below 1 μM.

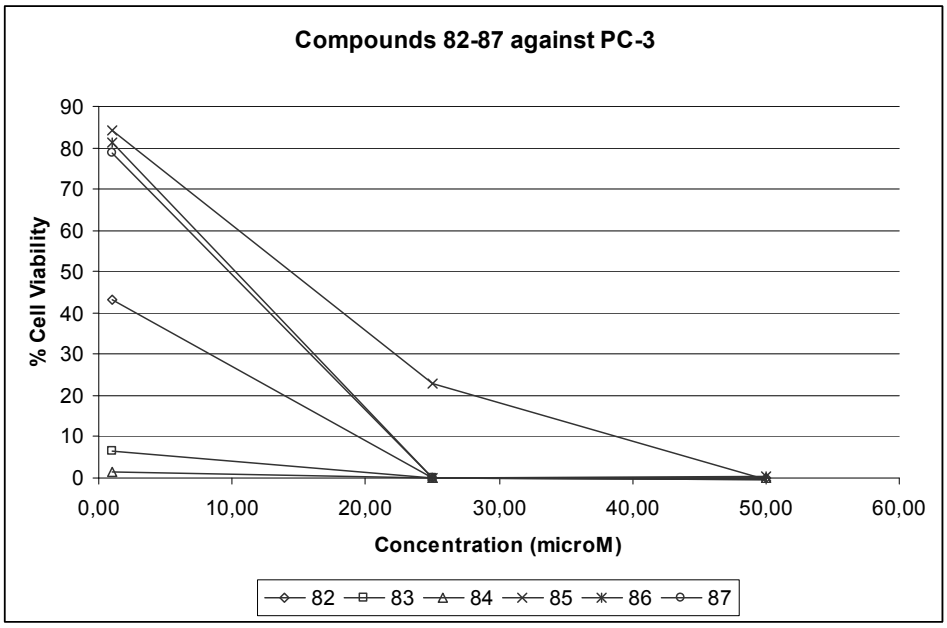


Figure 5.13. Concentration dependent cytotoxicities of compounds **82-87** against PC-3 cell lines for concentrations above 1μM.

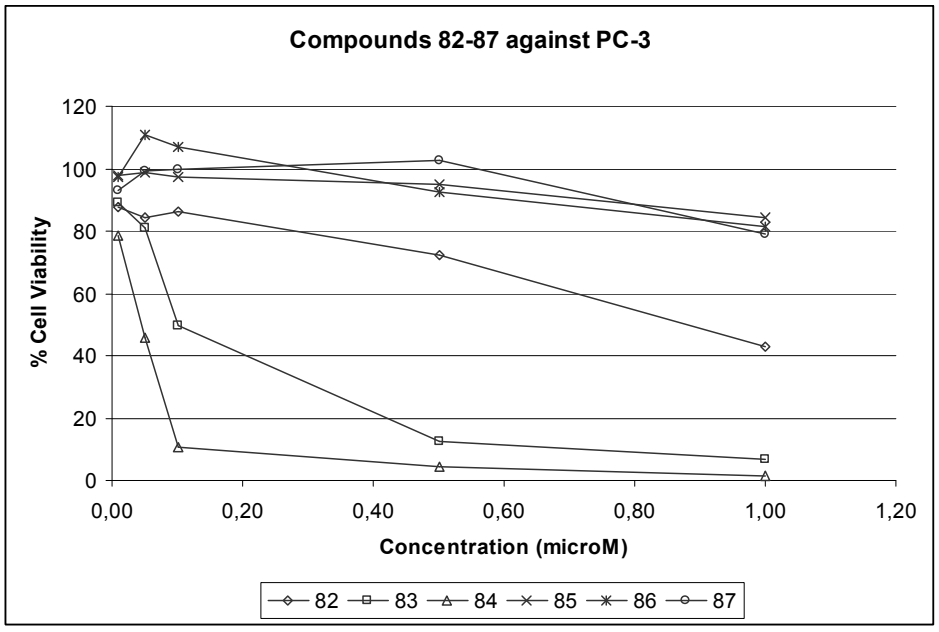


Figure 5.14. Concentration dependent cytotoxicities of compounds **82-87** against PC-3 cell lines for concentrations below 1μM.

Similarly, test results for MCF-7 cell lines have been shown in Figure 5.15 and 5.16 for concentrations above 1μM and for concentrations below 1 μM, respectively.

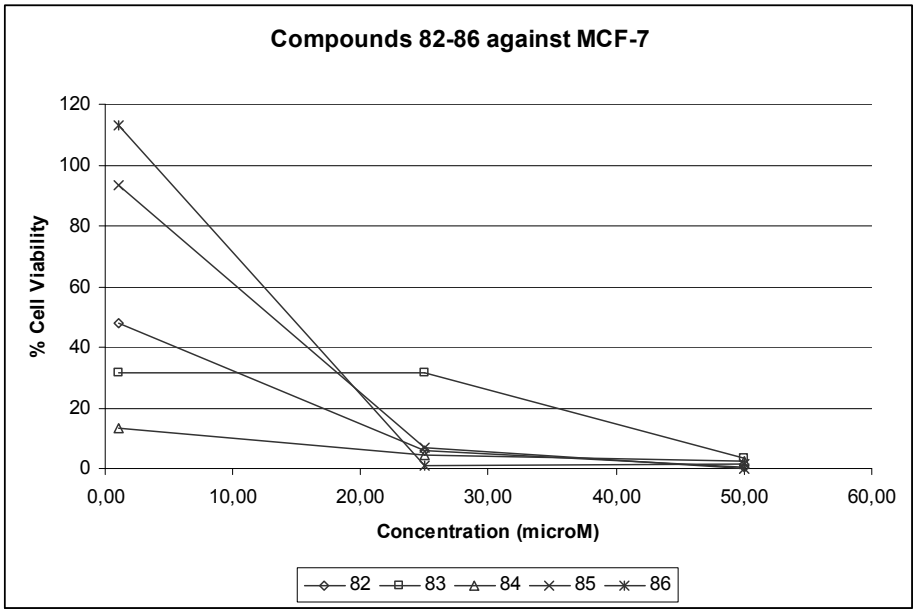


Figure 5.15. Concentration dependent cytotoxicities of compounds **82-86** against MCF-7 cell lines for concentrations above 1µM.

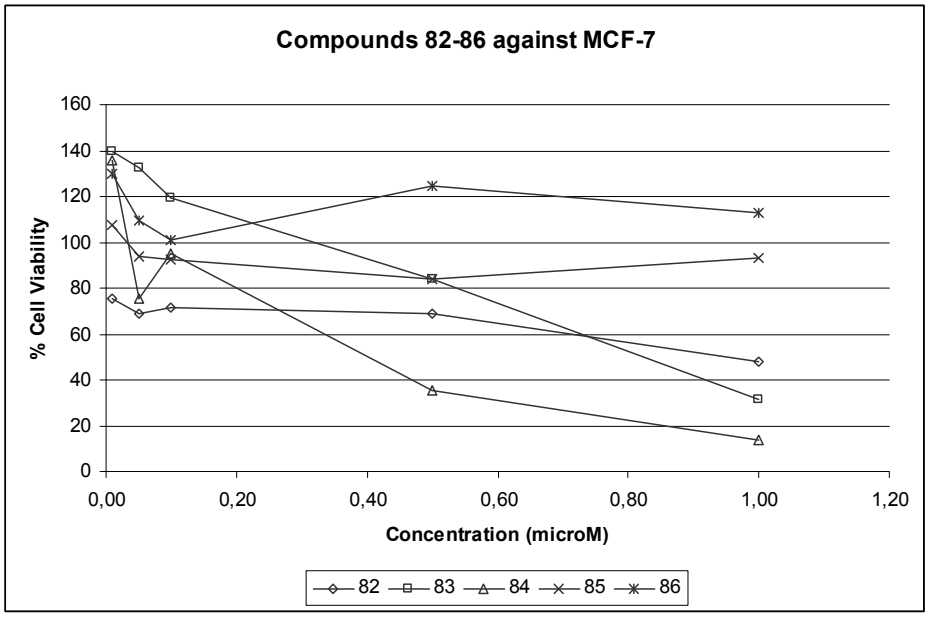


Figure 5.16. Concentration dependent cytotoxicities of compounds **82-86** against MCF-7 cell lines for concentrations below 1µM.

Form Figures 5.13, 5.14, 5.15, 5.16, it was observed that, tested compounds show similar trends in both cancer cell lines. However, compounds **82**, **85** and **89** have

relatively higher cytotoxicity than the others in both cell lines. IC_{50} values of these three compounds is below 1 μM for PC-3 and MCF-7 cell lines. (Figure 5.14 and 5.16)

On the other hand, cytotoxicity trend for these three compounds is different for both cell lines. But still compound **84** is a promising compound which has IC_{50} values 47 nM for PC-3 cell lines and ~400nM for MCF-7 cell lines. The next promising candidate is compound **83** which has IC_{50} values 100 nM for PC-3 and 900 nM for MCF-7 cell lines.

Beside these, in some of the figures the reason for the % cell viability higher than 100% can be resulting from two reasons (Figure 5.9, 5.10, 5.14, 5.15, 5.16). Either the compound itself has no significant activity below 1μM concentration and the number of cell in the wells at the beginning were not exactly the same, so the cells in the wells containing inactive molecules, may proliferate much more than the refence cell. Another possibility is the compound itself may cause the proliferation for concentrations below 1μM.

Considering activity of acrylate esters, which does not imply any reasonable structure activity relationship, but 2-pyranone derivatives **82**-**87** may have structure activity relationship. It is possible to say that 1-naphthyl substituted pyranones have higher cytotoxicities, and any steric hindrance at ring B makes them more active. It might be postulated that steric effect plays important role for the activity other than the restriction of rotations around the sigma bond of styrene. There may be no structure activity relationship in terms of activity between acrylate derivatives and pyranones.

CHAPTER 6

CONCLUSION

In this study, eight new possible anti-cancer drug candidate were proposed, synthesized and evaluated for their cytotoxicity against various cancer cell lines.

Design of the structure has been done on the basis of the knowledge about the structure activity relationship (SAR) studies of (R)- and (S)-goniothalamin derivatives. Compounds **75**, **76** and **86** were originally planned to show the effect of the rotation around the sigma bond in styrene sub-unit over the cytotoxic property. For the case compound **75**, it was observed that restriction of the rotation gave slightly more cytotoxic compound. (R)-configuration in the lactone ring seems to be crucial for strong activity.

To clarify the obtained conclusion, new bicyclic aryl and heteroaryl substituted analogues were synthesized. Among those, 1-naphthyl-substituted pyran-2-ones were the most cytotoxic which gave the clue about the structure activity relationship of the tested compounds. Compound **92**, structurally similar to acyl and amino acid substituted goniothalamin derivatives, also showed weak anti-tumor activity against PC-3 and MCF-7 cell lines. Another conclusion is steric factors around the pyran-2-one ring may help to increase the cytotoxic property of 1-naphthyl-substituted pyran-2-ones.

Acryloyl esters of allylic alcohol, intermediates in the synthetic route show also antiproliferative property but we could not observe any structure activity relationship. On the other hand, some of the acryloyl esters were effective as much as their lactone forms. 3-Quinoyl and 4-quinoyl acrylate derivatives (**85** and **86**) have comperatively similar anti-proliferative effect against both cancer cell lines as much as their lactone products (**103** and **104**).

Asymmetric synthesis of the compounds have been accomplished according to the previously described (R)-goniothalamin synthesis protocol (Fatima, et al. 2005). Although the synthesis route is straightforward, catalytic asymmetric allylation of aldehydes in the first step plays an important route for the synthesis.

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APPENDIX A

PURITY OF COMPOUNDS 96, 102 AND 84 BY HPLC (Chiral Column)

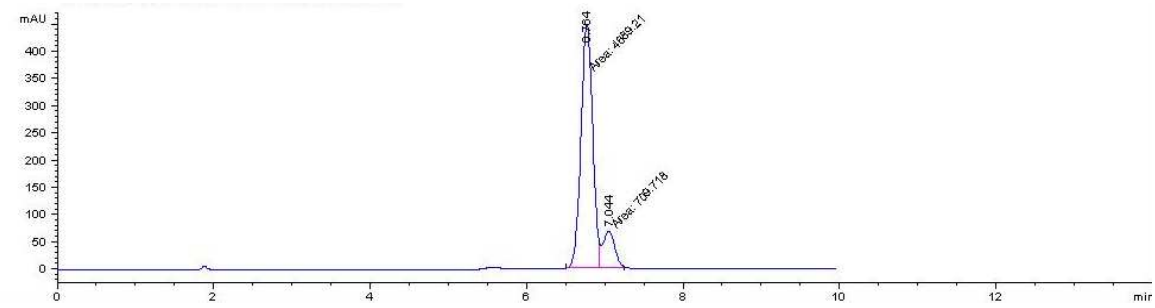

Figure A.1. Chiral HPLC chromatogram of compound **96** (DAD, Sig=210 nm)

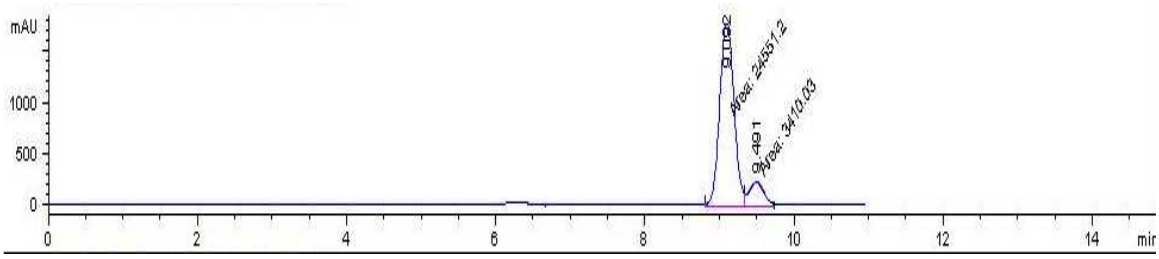

Figure A.2. Chiral HPLC chromatogram of compound **102** (DAD, Sig=210 nm)

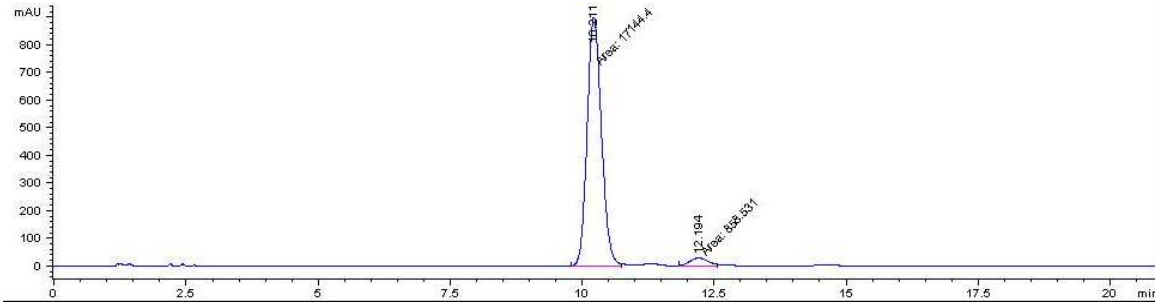

Figure A.3. Chiral HPLC chromatogram of compound **84** (DAD, Sig=210 nm)

APPENDIX B.

^{1}H, and ^{13}C-NMR SPECTRUM OF COMPOUNDS 96, 102 and 84

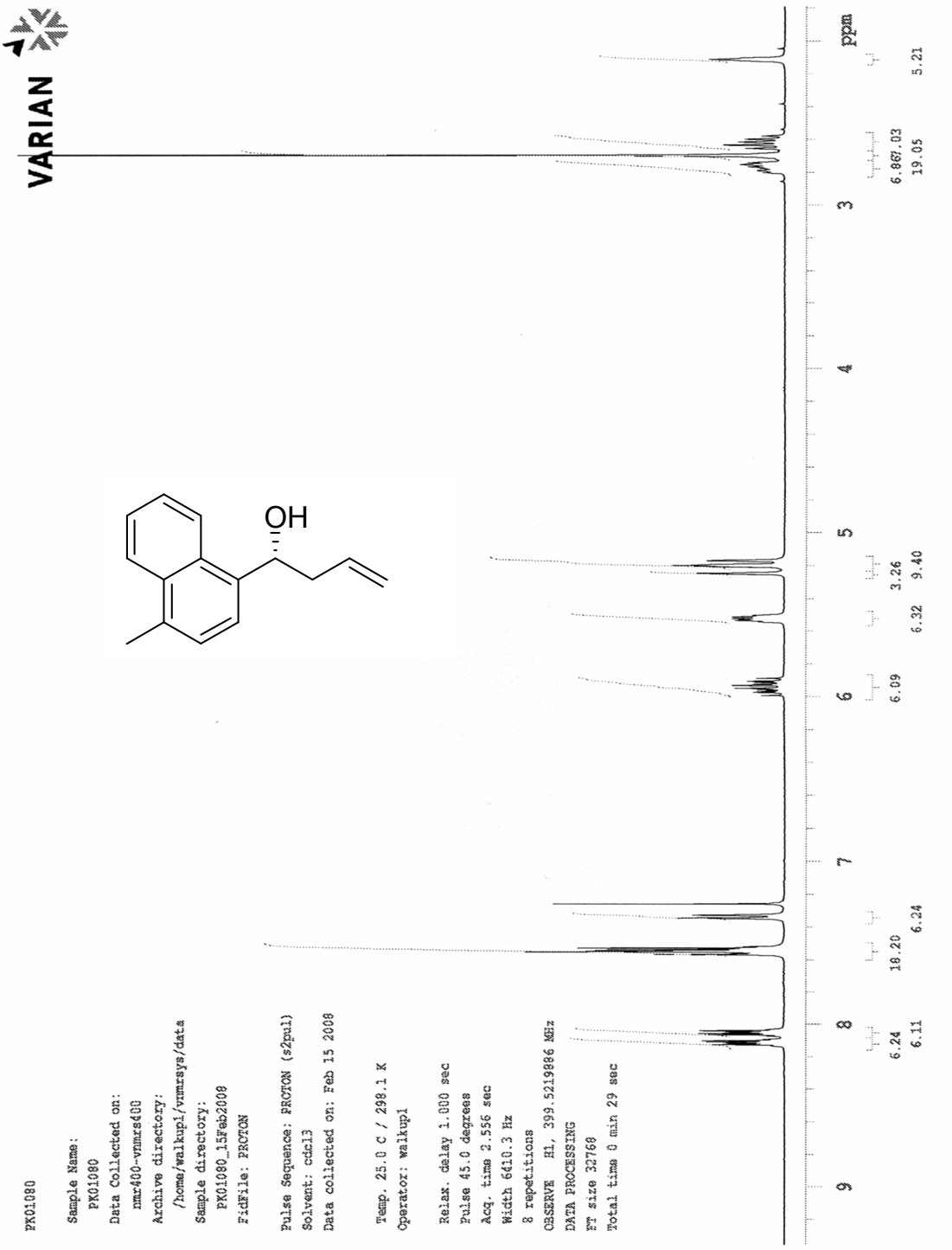

Figure B.1. ^{1}H NMR spectra of R-(+)-1-(4-methylnaphthalen-1-yl)-but-3-en-1-ol (**96**)

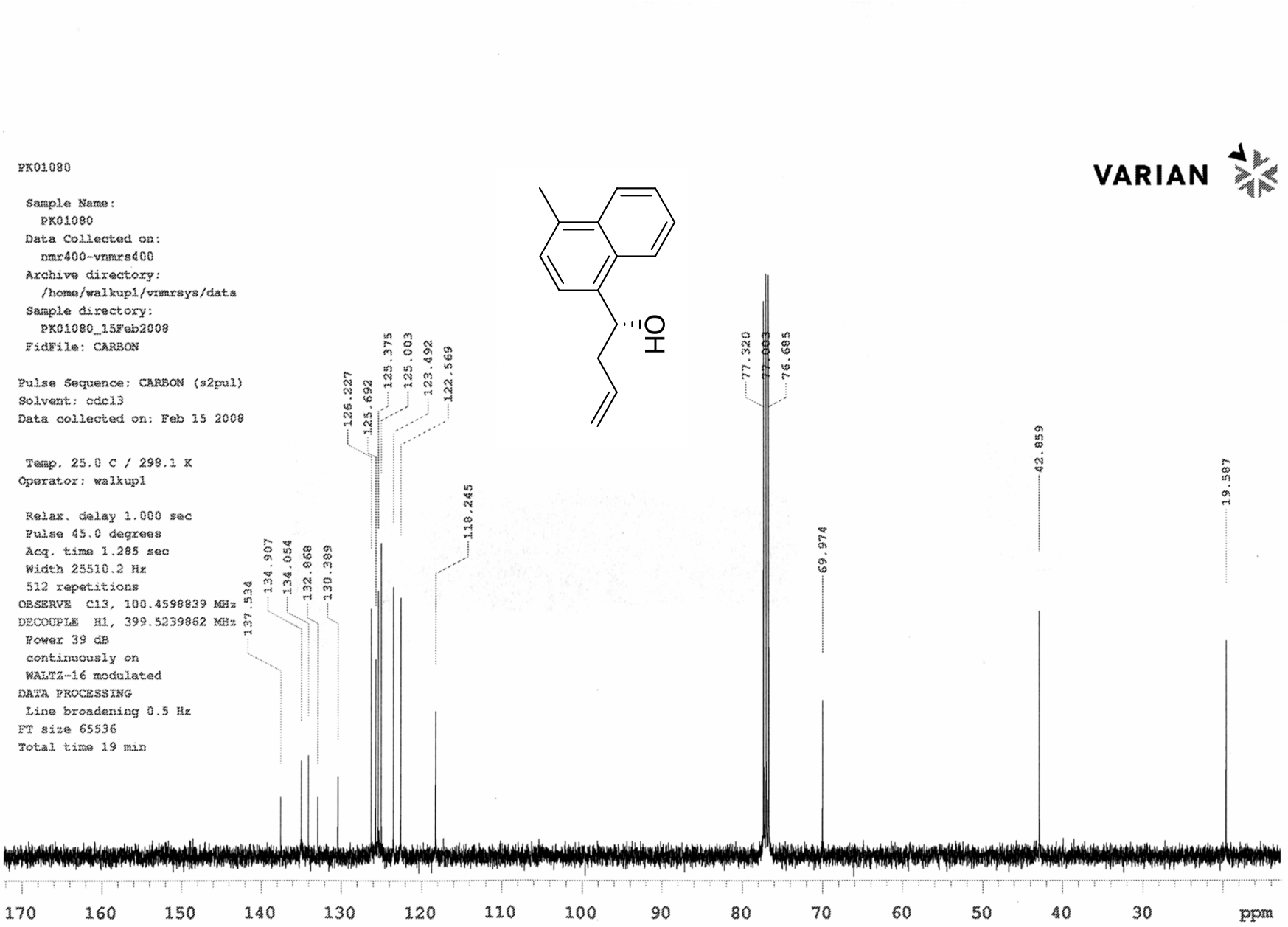

Figure B.2. ^{13}C NMR spactra of R-(+)-1-(4-methylnaphthalen-1-yl)-but-3-en-1-ol (**96**)

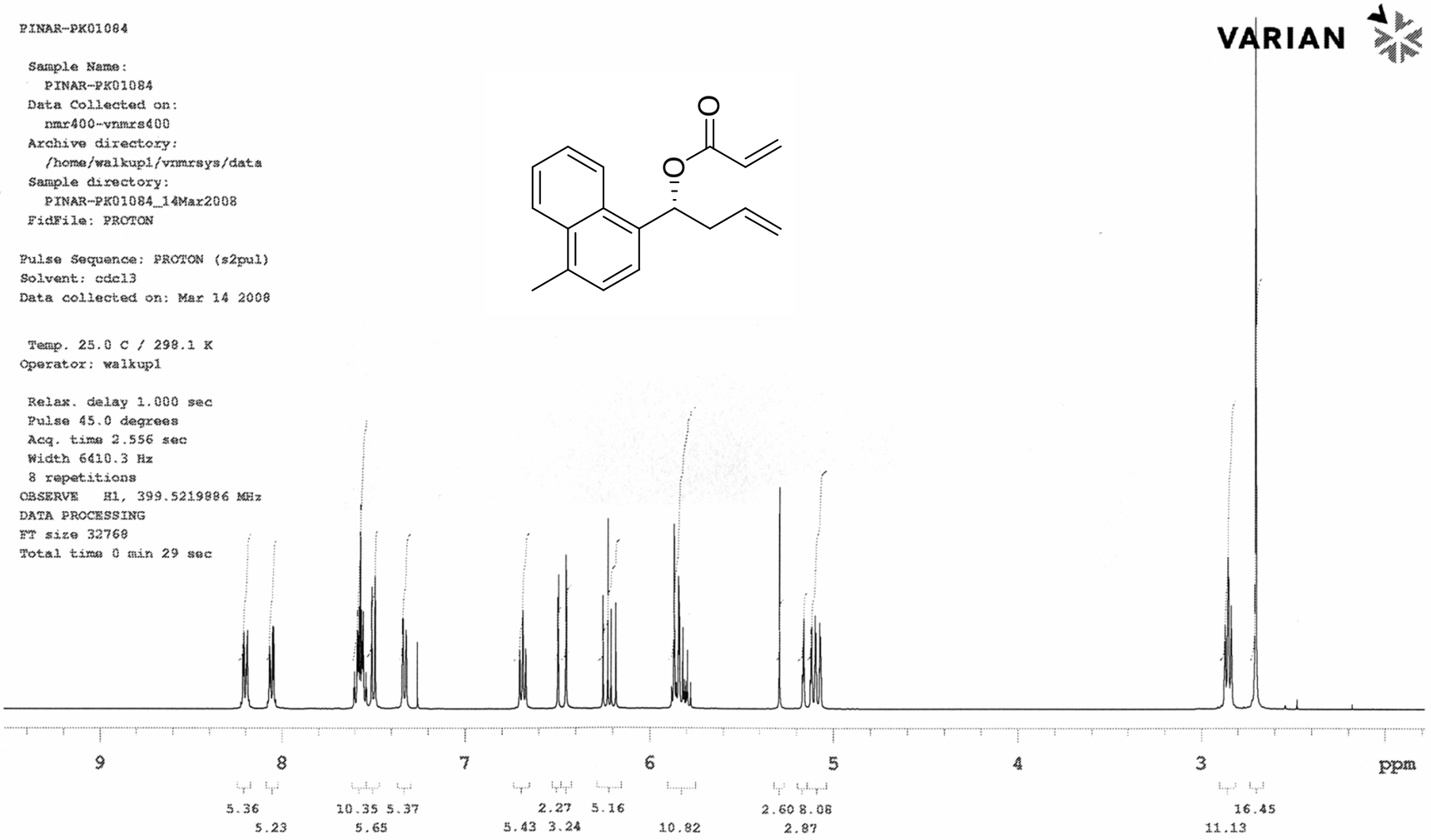

Figure B.3. ^{1}H NMR spectra of R-(+)-1-(4-methylnaphthalen-1-yl)-but-3-enyl-acrylate (**102**)

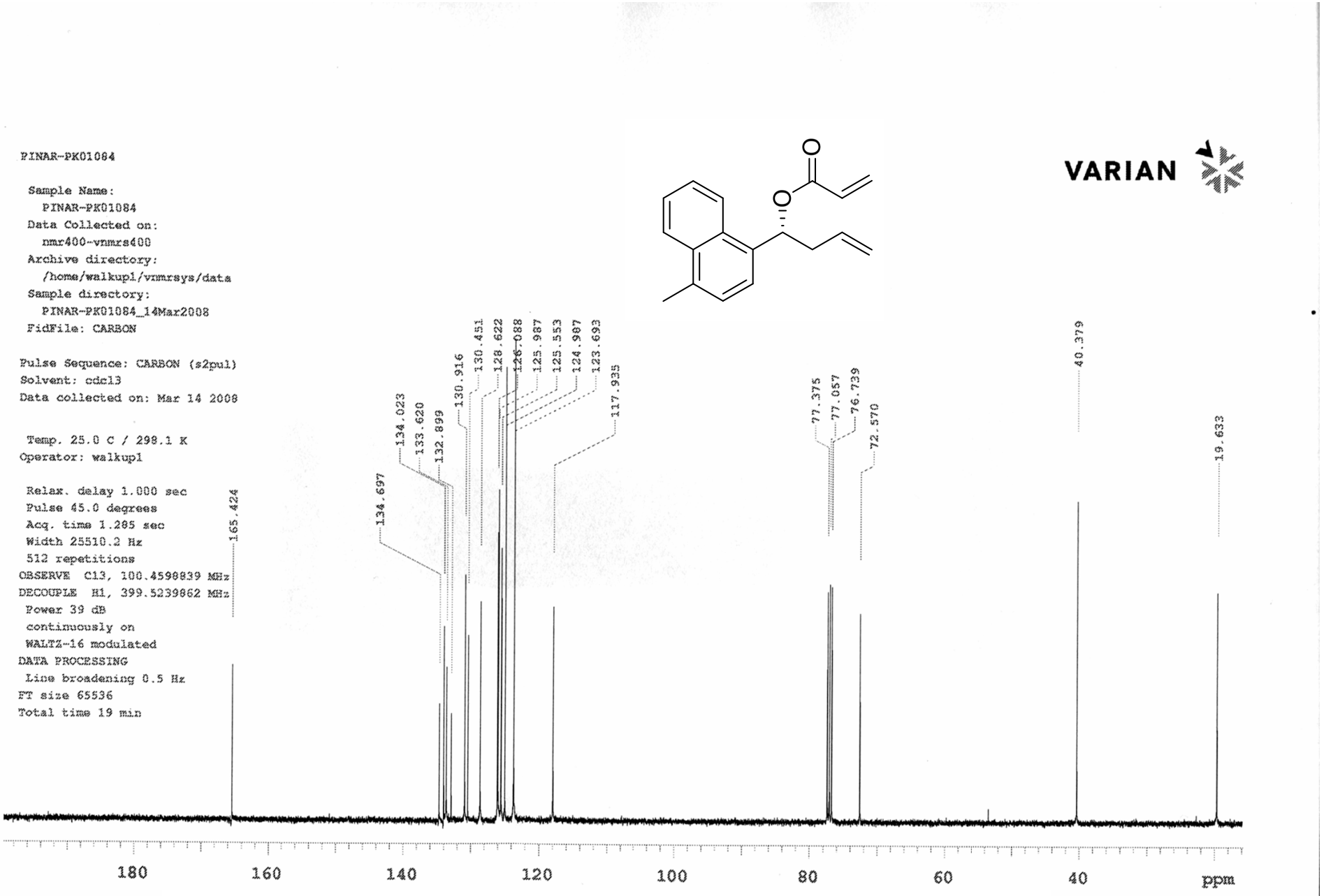

Figure B.4. ^{13}C NMR spectra of R-(+)-1-(4-methylnaphthalen-1-yl)-but-3-enyl-acrylate (**102**)

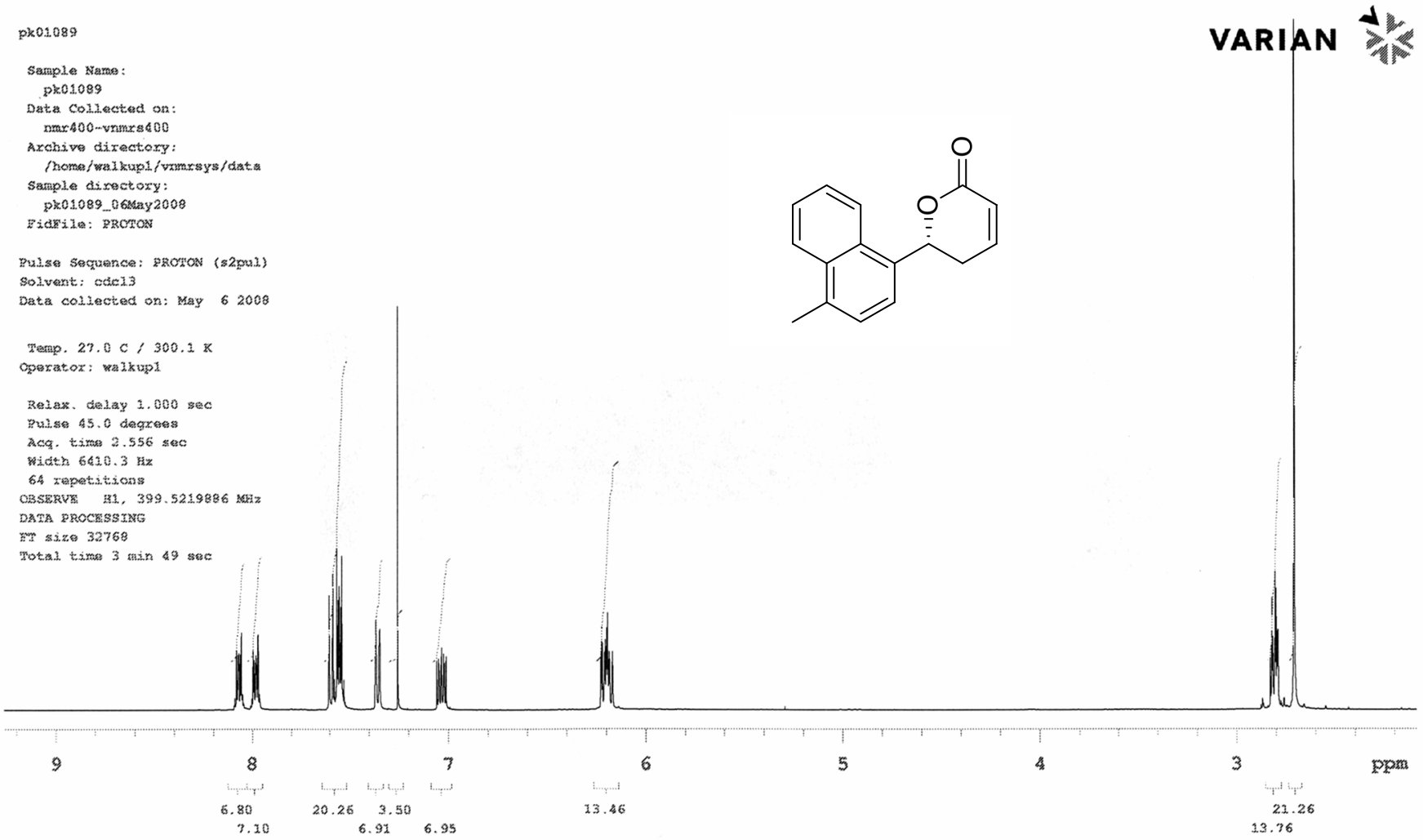

Figure B.5. ^{1}H NMR spectra of R-(+)-1-(4-methylnaphthalen-1-yl)-5,6-dihydro-2H-pyran-2-one (**84**)

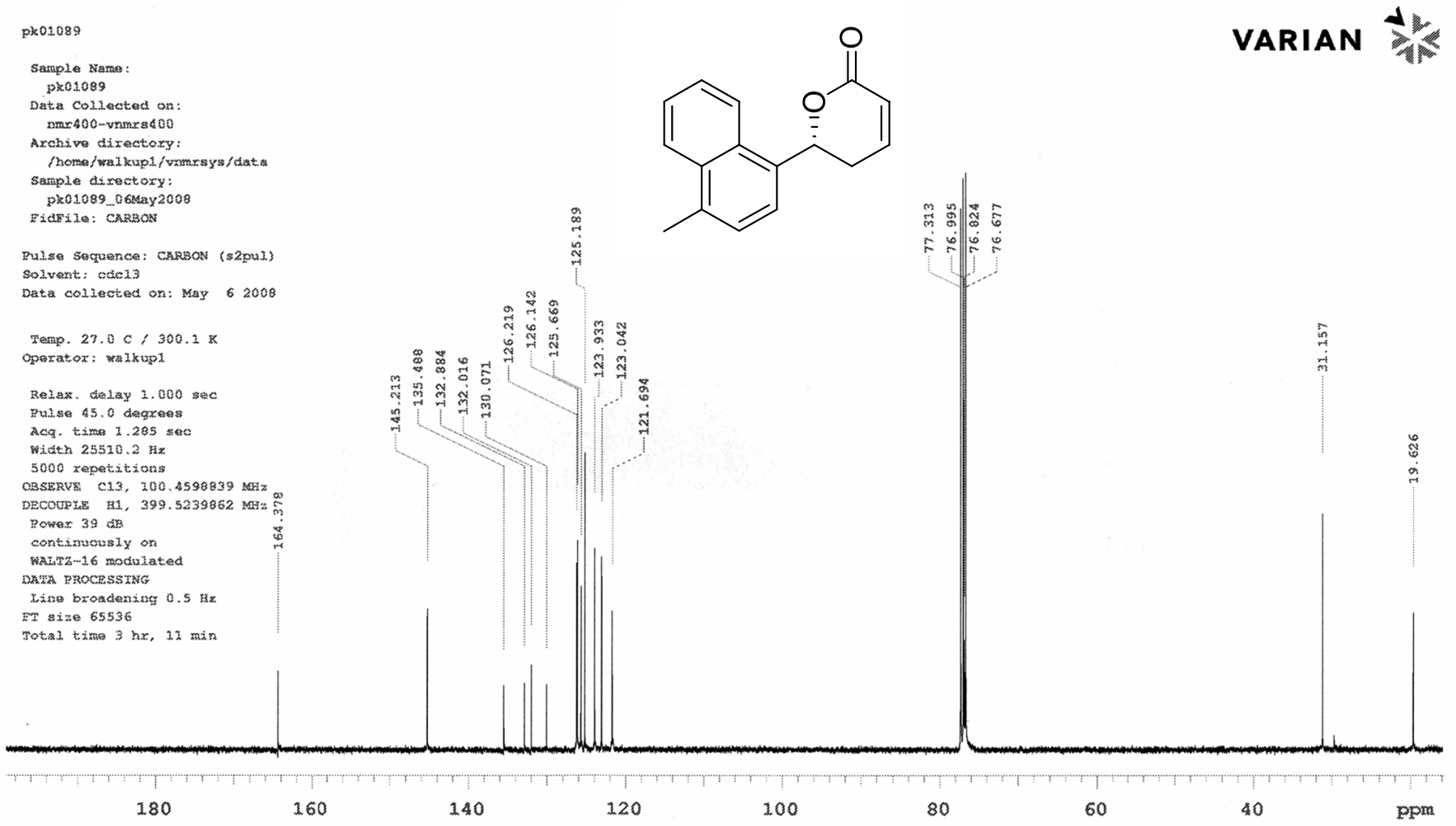

Figure B.6. ^{13}C NMR spectra of R-(+)-1-(4-methylnaphthalen-1-yl)-5,6-dihydro-2H-pyran-2-one (**84**)