T.R.
SAKARYA UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

SYNTHESIS OF (-)-CAMPHENE DERIVATIVES AND EVALUATION OF THEIR BIOLOGICAL ACTIVITIES

MSc. THESIS

Ansam ALMOTORY

Chemistry Department

Organic Chemistry Program

MARCH 2024

T.R.
SAKARYA UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

SYNTHESIS OF (-)-CAMPHENE DERIVATIVES AND EVALUATION OF THEIR BIOLOGICAL ACTIVITIES

MSc. THESIS

Ansam ALMOTORY

Chemistry Department

Organic Chemistry Program

Thesis Advisor: Prof. Dr. Ahmet TUTAR

MARCH 2024

The thesis work titled “SYNTHESIS OF (-)-CAMPHENE DERIVATIVES AND EVALUATION OF THEIR BIOLOGICAL ACTIVITIES” prepared by Ansam ALMOTORY was accepted by the following jury on 01/03/2024 by unanimously/majority of votes as a MSc. THESIS in Sakarya University Graduate School of Natural and Applied Sciences, Chemistry department, Organic Chemistry program.

Thesis Jury

Jury Member :	**Prof. Dr. Ahmet TUTAR** (Advisor) Sakarya University	**.....**
Jury Member :	**Prof. Dr. Fatih SÖNMEZ** Sakarya University of Applied Sciences	**.....**
Jury Member :	**Dr. Öğr. Üyesi Barış Seçkin ARSLAN** Sakarya University	**.....**

STATEMENT OF COMPLIANCE WITH THE ETHICAL PRINCIPLES AND RULES

I declare that the thesis work titled "Synthesıs of (-)-Camphene Derivatives and Evaluation of Their Biological Activities", which I have prepared in accordance with Sakarya University Graduate School of Natural and Applied Sciences regulations and Higher Education Institutions Scientific Research and Publication Ethics Directive, belongs to me, is an original work, I have acted in accordance with the regulations and directives mentioned above at all stages of my study, I did not get the innovations and results contained in the thesis from anywhere else, I duly cited the references for the works I used in my thesis, I did not submit this thesis to another scientific committee for academic purposes and to obtain a title, in accordance with the articles 9/2 and 22/2 of the Sakarya University Graduate Education and Training Regulation published in the Official Gazette dated 20.04.2016, a report was received in accordance with the criteria determined by the graduate school using the plagiarism software program to which Sakarya University is a subscriber, I have received an ethics committee approval document. I accept all kinds of legal responsibility that may arise in case of a situation contrary to this statement.

01/03/2024

Ansam ALMOTORY

I dedicate my thesis to my mother (God mercy her)

ACKNOWLEDGMENTS

I would first like to thank my advisor, Prof. Dr. Ahmet TUTAR, for allowing me to work with him. I felt at my country during my time in Türkiye because of your encouragement, and never-ending offer of new chances. Additionally, I want to thank the department head and the other committee members for their support. I also thank my laboratory colleagues Yavuz DERİN and Büşra ALBAYRAK MISIR who stood by me at every stage for their accuracy and interest in obtaining NMR spectra of composite vehicles. A heartfelt acknowledgment is owed to Dr. Raşit Fikret YILMAZ from the Fundamental Sciences Department of the National Defense University Naval Academy. Without his assistance and support, I would not have been able to complete my research. I would like to thank Assoc. Prof. Ali AYDIN for carrying out the biological activity studies. I have always valued my family's support and will always be grateful for all they have done and will continue to do for me. I want to thank all my friends at this point.

Ansam ALMOTORY

TABLE OF CONTENTS

Page

ABBREVIATIONS

^{1}H NMR	: Proton-nuclear magnetic resonance spectroscopy
^{13}C NMR	: Carbon 13-nuclear magnetic resonance spectroscopy
FTIR	: Infrared spectroscopy
g	: Gram
mL	: Milliliter
mmol	: Millimolar
ppm	: Part per million
MHz	: Megahertz
h	: Hour
α	: Alfa
β	: Beta
dd	: Doublet in doublets
m	: Multiplet
s	: Singlets

LIST OF TABLES

Page

LIST OF FIGURES

Page

SYNTHESIS OF (-)-CAMPHENE DERIVATIVES AND EVALUATION OF THEIR BIOLOGICAL ACTIVITIES

SUMMARY

Terpenes are aromatic oils that give their fragrance and taste. These compounds have additionally been acknowledged to hold an array of medicinal advantages, (±)-camphene is a one of example cannabis terpene possessing a strong musky and earthy smell with hints of pine. (±)-Camphene is a primary ingredient in many perfumes, oils, and topical lotions. It is also naturally present in conifers, nutmeg, ginger, and rosemary. A common synthetic method for (±)-camphene is to use pinene, another cannabis terpene. (±)-Camphene is commonly used in some of salves, creams and lotions that are cannabis-based and its possibility of this ability to treat skin conditions such as eczema and psoriasis indicates camphene could hold antifungal and antibacterial effectiveness.

Camphene is generally more commonly found in indica strains with medicinal potential and is notable. In this study, (-)-camphene (70) will be used as key compounds for the synthesis of new biologically active compounds (73-77). Starting from (-)-camphene [75% (camphene with 90% fenchene)], bromination reactions and Suzuki-Miyaura coupling reactions will be carried out. In this bromination system, (-)-camphene (70) will be dissolved in dichloromethane (20 mL), and Br_2 (dissolved in CH_2Cl_2) will be added dropwise to the reaction mixture with the help of a pressure balanced dropping funnel. When aniline is added to dibromo-(-)-camphene (71) and the reaction mixture is boiled under reflux for 2 hours, (1*S*,4*R*)-3-(Bromomethylene)-2,2-dimethylbicyclo-2,2-dimetilbisiklo[2.2.1]heptane (72) will be obtained.

Brominated derivatives suitable for metal-catalyzed reactions are an important key compound for many new products. In this study, it is planned to apply metal-catalyzed Suzuki-Miyaura coupling reactions for the synthesis of new (-)-camphene derivatives from monobromo-(-)-camphene. Coupling reactions of bicyclic structures were carried out in the presence of catalysts such as $Pd(PPh_3)_4$ and various boronic acids (phenyl, 4-methoxyphenyl, 4-thiomethylphenyl, 4-ethylphenyl, 4-trifluoromethoxyphenyl). Following the general synthesis procedure for the synthesis of relevant compounds, monobromo-(-)-camphene (72) and related boronic acid compounds (73-77) were interacted with, then K_2CO_3 was added to the reaction mixture as a base, toluene ethanol and water as solvents (1:1:1) was added and boiled under reflux at 110°C for 17 hours. Then, the crude product was extracted with ethylacetate (3x50 mL) and the organic phase was dried with Na_2SO_4 and the reaction mixture was purified by silica gel column chromatography. The final compounds were characterized using ^{1}H NMR, ^{13}C NMR and FTIR spectroscopy methods; they were also shown to be photoactive and negatively polarimetric.

The biological effects of (-)-camphene derivatives (cancer cell lines and cell culture, cytotoxicity test, DNA fragmentation by agarose gel electrophoresis, migration assay, antibacterial and antifungal activity measurement by microdilution assay) were examined. In this study, A549 (ATCC, CCL-185), Calu1 (ATCC, HTB-54), and

H1650 (ATCC, CRL-5883) lung cancer cell lines, SW1353 (ATCC, HTB-94)/MG63 (ATCC, CRL-1427)/, and Saos2 (ATCC, HTB-85) bone cancer cell lines], Beas2B (RRID, CVCL-0168) normal lung cell line, and HC (Sigma Aldrich, 402-05A) normal chondrocytes cell line were used. MTT test was used to measure the effects of the synthesized compounds (71-77) on cell proliferation and NCI-60 survival parameter values. It was determined by the LDH method whether the compounds to be tested (71-77) were cell cytotoxic or cytostatic. DNA laddering activity of the (-)-camphene compounds was evaluated by a DNA laddering assay in accordance with the standard method. Wound healing assay was used to determine the effect of the compounds on the migration of cells. It is a method based on measuring the proliferation ability of cells in the medium containing the test substance. For measuring the antibacterial and antifungal activities using microdilution test in this project [*E. faecalis* **VRE** ATCC 19433, *E. faecalis* ATCC 29212, *S. aureus* **MSSA** ATCC 29213, *S. aureus* ATCC 25923, *S. aureus* **MRSA** ATCC 46300, *E. coli* ATCC 25922, *P. aeruginosa* **AGME** ATCC 27853, *C. albicans* (ATCC 10231), *S. gordonii* (NCTC 7870) and *A. actinomycetemcomitans* (ATCC 33384)] were used. Therefore, these molecules lack antimicrobial properties against all bacteria and fungi but they have the effect of (-)-camphene molecules are partly responsible for their anticancer activities it may be a good option for cancer treatment and compound 72 was more cancer-specific for bone and lung cancer types, and make it is the most convenient one for further preclinical studies.

(-)-KAMFEN TÜREVLERİNİN SENTEZİ VE BİYOLOJİK AKTİVİTELERİNİN DEĞERLENDİRİLMESİ

ÖZET

Terpenler, koku ve tatlarını veren aromatik yağlardır. Bu bileşiklerin ayrıca çeşitli tıbbi faydalar sağladığı da kabul edilmiştir. (±)-Kamfen, çam terpeni olarak bilinen bir cannabis terpeninin güçlü bir misk ve toprak kokusuna sahip bir örneğidir. Kamfen $C_{10}H_{16}$ kimyasal formülüne sahiptir ve molekül ağırlığı 136,24 g/mol'dür. Kamfen, D-kamfen ve L-kamfen olarak da bulunur. Kamfen katı bir görünüme sahiptir ancak erime noktası 50°C'dir. (±)-Kamfen suda çözünmezken sikloheksan, alkol ve kloroform içeren doğal çözücülerde kolaylıkla çözünür. Toksafen ve kafurun her ikisine de kamfen denir. İzobornil asetat gibi tatlandırıcılar ve aromatikler ve diğer kimyasalları oluşturan organik bileşikler ara madde olarak işlev görür. 1800'lü yıllarda yakıt olarak gaz yağı yerine kamfen kullanılmıştır. (±)-Kamfen birçok parfüm, yağ ve topikal losyonun ana bileşenidir. Ayrıca kozalaklı ağaçlarda, hindistan cevizi, zencefil ve biberiye gibi bitkilerde doğal olarak bulunur. (±)-Kamfen için yaygın bir sentetik yöntem, başka bir kenevir terpeni olan pinen kullanmaktır. (±)-Kamfen, kenevir bazlı bazı merhemlerde, kremlerde ve losyonlarda yaygın olarak kullanılır ve kamfenin antifungal ve antibakteriyel etkinliği sayesinde egzama ve sedef hastalığı gibi cilt hastalıklarını tedavi etme yeteneğine sahip olduğunu gösterir.

Terpenler birçok bitki ve hayvanda bulunan geniş ve çeşitli bir organik bileşik sınıfıdır. Terpenler karakteristik aroma ve tatlarıyla bilinirler ve aynı zamanda çeşitli biyolojik işlevlere sahiptirler. Terpenler birçok reaksiyon için uygun yapıya sahip bileşiklerdir. Terpenler, organik sentez kimyasında önemli rolü olan oksidasyon, izomerizasyon, polimerizasyon, hidrojenasyon ve indirgenme reaksiyonlarını verirler. Ayrıca kamfen gibi bisiklik monoterpenlerde Wagner-Meervein yeniden düzenlemesi gerçekleşebilir. Bisiklik monoterpenlerin düzenlenmesi yıllardır ilgi odağı olmuştur ve hala bisiklik monoterpenlerde iyonik katılma reaksiyonları Wagner-Meerwein yeniden düzenlenmesi nedeniyle meydana gelmektedir. Bisiklik monoterpenlerdeki Wagner-Meerwein yeniden düzenlemesi, metil grubu gibi bir sübstitüentin çift bağ üzerindeki bir karbon atomundan tek bağ üzerindeki bitişik bir karbon atomuna geçişini içerir. Bu sürece genellikle rezonans veya komşu gruplar tarafından stabilize edilebilen bir karbokatyon ara ürününün oluşumu eşlik eder. Wagner-Meerwein kuralları, karbokatyon ara maddesinin stabilitesine dayalı olarak yeniden düzenlemenin sonucunu tahmin eder.

Bu çalışmanın en özgün yanı bisiklik yapıya sahip kamfenin brominasyonu ve bu brominasyon esnasında düzenlenmenin durdurulmasıdır. Bisiklik yapıdaki rijit alkenlerin bromlanma reaksiyonları klasik alkenlerin bromlanma reaksiyonlarından oldukça farklıdır. Normal şartlar altında brominasyon reaksiyonları bromonyum iyonu üzerinden yürürken, bu tür moleküllerin brominasyonunda çoğunlukla bromonyum iyonu non-klasik karbokatyona göre düzenlenir. Bu sebeple brominasyon non-klasik karbokatyon üzerinden ilerler. Klasik ve non-klasik karbokatyon arasındaki en temel fark, klasik karbokatyonların üç kimyasal bağda altı elektrona sahip bir karbon

atomuna sahip olması,non-klasik karbokatyonların ise üç merkezli, iki elektronlu yapısının olmasıdır. İyon geçişi, bisikloheptan iskelet yapısına sahip olan klasik ve non-klasik karbokatyonların (±)-kamfen hidroklorürün izobornil klorüre yeniden düzenlenmesi sırasında oluşur. Bu çalışmalardan sonra devam eden araştırmalarda Meervein kimyasal reaksiyonlardaki bu tür ürünleri karbonyum olarak tanımlamıştır. Farklı bir araştırmada 2-norbornil tosilatın solvolizi sırasında oluşan ara ürünler non-klasik karbokatyonlar olarak tanımlanmıştır.

Kenetlenme reaksiyonları organik kimyanın vazgeçilmez ve temel reaksiyonlarından biridir. Çalışmalarımızın büyük bir kısmını brominasyon reaksiyonları yanında Suzuki-Miyaura çapraz kenetlenme reaksiyonları oluşturmaktadır. Organik kimyada karbon-karbon bağlarının oluşumu için genellikle Suzuki-Miyaura çapraz kenetlenme reaksiyonları ile gerçekleştirilebilir. Organoboronik asit ile halojenürler veya triflat arasında Suzuki-Miyaura çapraz kenetlenme reaksiyonları için bir paladyum katalizörünün kullanılması gerekir. Pd ve Ni gibi geçiş metalleri tipik olarak katalizör olarak kullanılır. "Pd(0)-Pd(II) katalitik çevrimi"ni kullanan bu geleneksel reaksiyonun mekanizması, oksidatif katılma, transmetalasyon ve redüktif eliminasyon adımlarından oluşmaktadır. Hem çevre dostu hem de basit olan Suzuki-Miyaura çapraz kenetlenme reaksiyonları poliolefinleri, stirenleri ve sübstitüe edilmiş bifenilleri sentezlemek için kapsamlı bir şekilde araştırılmış ve sıklıkla uygulanmıştır.

(±)-Kamfen monosikloterpenlerin bir çeşididir. Kamfen genellikle tıbbi potansiyele sahip indika türlerinde daha yaygın olarak bulunur ve dikkate değerdir. Bu çalışmada (-)-kamfen (70), yeni biyolojik olarak aktif bileşiklerin (73-77) sentezi için anahtar bileşikler olarak kullanılacaktır. (-)-Kamfenden [%75 (%90 fenchenli kamfen)] başlayarak brominasyon reaksiyonları ve Suzuki-Miyaura kenetlenme reaksiyonları gerçekleştirilecektir. Bu brominasyon sisteminde, (-)-kamfen (70), diklorometan (20 mL) içinde çözülerek, Br_2 (CH_2Cl_2 içinde çözünmüş), bir basınç dengeli damlatma hunisi yardımıyla reaksiyon karışımına damla damla eklenecektir. Dibromo-(-)-kamfene (71) anilin eklenip reaksiyon karışımı 2 saat boyunca geri soğutucu altında kaynatıldığında (1*S*,4*R*)-3-(Bromometilen)-2,2-dimetilbisiklo[2.2.1]heptan (72) elde edilecektir.

Metal katalizli reaksiyonlar için uygun bromlu türevler birçok yeni ürün için önemli bir anahtar bileşiktir. Bu çalışmada metal katalizli Suzuki-Miyaura kenetlenme reaksiyonları, monobromo-(-)-kamfenden yeni (-)-kamfen türevlerinin sentezi için uygulanması planlanmaktadır. Bisiklik yapıların kenetlenme reaksiyonları $Pd(PPh_3)_4$ gibi katalizörler varlığında ve çeşitli boronik asitler (fenil, 4-metoksifenil, 4-tiyometilfenil, 4-etilfenil, 4-triflorometoksifenil) ile gerçekleştirilmiştir. İlgili bileşikler sentezi için genel sentez prosedürü izlenerek monobromo-(-)-kamfen (72) ve ilgili boronik asit bileşikler (73-77) ile etkileştirilmiş, ardından reaksiyon karışımına baz olarak K_2CO_3, çözücü olarak toluen etanol ve su (1:1:1) eklenip 110°C'de 17 saat boyunca geri soğutucu altında kaynatılmıştır. Daha sonra ham ürün etilasetat (3x50 mL) ile ekstrakte edildi ve organik faz Na_2S0_4 ile kurutuldu ve reaksiyon karışımı silikajel kolon kromatografisiyle saflaştırıldı. Nihai bileşikler, ^1H NMR, ^{13}C NMR ve FTIR spektroskopi yöntemleri kullanılarak karakterize edildi; ayrıca fotoaktif ve negatif polarimetrik oldukları da gösterildi.

(-)-Kamfen türevlerinin biyolojik etkileri (kanser hücre hatları ve hücre kültürü, sitotoksisite testi, agaroz jel elektroforezi ile DNA fragmentasyonu, migrasyon deneyi, mikrodilüsyon deneyi ile antibakteriyel ve antifungal aktivite ölçümü) incelendi. (-)-Kamfen türevlerinin biyolojik etkileri (kanser hücre hatları ve hücre kültürü,

sitotoksisite testi, agaroz jel elektroforezi ile DNA fragmentasyonu, migrasyon deneyi, mikrodilüsyon deneyi ile antibakteriyel ve antifungal aktivite ölçümü) incelendi. Bu çalışmada A549 (ATCC, CCL-185), Calu1 (ATCC, HTB-54) ve H1650 (ATCC, CRL-5883) akciğer kanseri hücre hatları, SW1353 (ATCC, HTB-94)/MG63 (ATCC, CRL-1427)/ ve Saos2 (ATCC, HTB-85) kemik kanseri hücre hatları], Beas2B (RRID, CVCL-0168) normal akciğer hücre hatları ve HC (Sigma Aldrich, 402-05A) normal kondrosit hücre hatları kullanıldı. Sentezlenen bileşiklerin (71-77) hücre çoğalması ve NCI-60 hayatta kalma parametresi değerleri üzerindeki etkilerini ölçmek için MTT testi kullanıldı. Test edilecek bileşiklerin (71-77) hücre sitotoksik mi yoksa sitostatik mi olduğu LDH yöntemiyle belirlendi. (-)-kamfen bileşiklerinin DNA merdivenleme aktivitesi, standart yönteme uygun olarak bir DNA merdivenleme tahlili ile değerlendirildi. Bileşiklerin hücrelerin göçü üzerindeki etkisini belirlemek için yara iyileşme denemeleri kullanıldı. Test maddesini içeren ortamdaki hücrelerin çoğalma yeteneğinin ölçülmesine dayanan bir yöntemdir. Bu projede mikrodilüsyon testi kullanılarak antibakteriyel ve antifungal aktivitelerin ölçülmesi için [*E. faecalis* **VRE** ATCC 19433, *E. faecalis* ATCC 29212, *S. aureus* **MSSA** ATCC 29213, *S. aureus* ATCC 25923, *S. aureus* **MRSA** ATCC 46300, *E. coli* ATCC 25922, *P. aeruginosa* **AGME** ATCC 27853, *C. albicans* (ATCC 10231), *S. gordonii* (NCTC 7870) ve *A. actinomycetemcomitans* (ATCC 33384)] kullanıldı. Bu nedenle bu moleküller tüm bakteri ve mantarlara karşı antimikrobiyal özelliklere sahip değildir ancak (-)-kamfen molekülleri bu özelliklere sahiptirler, antikanser aktivitelerinden kısmen sorumludurlar, kanser tedavisi için iyi bir seçenek olabilir ve bileşik 72 kemik kanseri için daha özgüdür ve akciğer kanseri türlerini daha ileri klinik öncesi çalışmalar için en uygun hale getirmektedir.

1. INTRODUCTION

Terpenes are aromatic oils that give their fragrance and taste. These molecular compounds have also been recognized to carry an array of medicinal advantages when blended with different cannabinoids and terpenes, the synergistic recuperation nature of cannabis simply takes impact, however, every terpene has specific traits of its very own (Quintans-Júnior et al., 2013).

(±)-Camphene is one of the cannabis terpenes naturally found in ginger, rosemary, conifer, and nutmeg it is often synthesized from pinene cannabis terpene. Also, a significant component of numerous scents, oils, and topical treatments. Research on its some of capacity to possibly cure psoriasis and eczema on the skin suggests that camphene it can have antibacterial and antifungal efficacy. (±)-Camphene is usually more prevalent in indica strains with noteworthy high medicinal potential (Hachlafi et al., 2023; Girola et al., 2015).

For many years, studies looked into (±)-camphene's like antioxidant quality as a potential treatment for lung inflammation, and it had a good role for regulating cardiovascular health furthermor it observed notable reductions in cholesterol and triglycerides, and in December 2012 (±)-camphene demonstrated potential as a potent antioxidant, pain reliever, and antiinflammatory (Martinez et al., 2012). In vivo, camphene showed effectiveness against cancer by impeding the growth of very aggressive (Hachlafi et al., 2023; Vallianou et al., 2011).

2. LITERATURE REVIEW

2.1. Terpenes and Their Importance

Terpenes (Greek: terebinths = tar wood, Latin; Pistacia terebinths) are classical. Terpenes are organic compounds that are naturally occurring in plants, including cannabis. They produce the distinct flavor and aroma of various cannabis strains as well as a variety of possible therapeutic effects. Terpenes are typically found in essential oils. Essential oils are often liquid, intensely scented, and flammable chemicals, industry uses for them include perfumery and cosmetics in general. Water vapor is dragging them along. Organic materials are highly soluble in solvents but insoluble in water. Every aspect of a plant, including its leaves, fruits, and flowers, may be found in its organs. More than 2000 essential oil constituents have been discovered thus far. Terpenes, or phenylpropanoids, are the most significant of them. Furthermore, volatile nitrogen and sulfur-containing molecules are also found to be present in many water vapors. The constituents of essential oils can normally be divided into four classes for analysis (aromatic substances, terpene substances, straight-chain hydrocarbons, sulfur-bearing, and nitrogen compounds) (Khaleel et al., 2018; Christianson, 2017; Trombetta et al., 2005). About 90% of the chemical components in essential oils are terpenes. One of the most popular categories of organic compounds is terpenes. Animals and plants both provide a variety of purposes, but they are also crucial for adding flavor to meals. Citrus, cinnamon, and other spice flavors, for instance, are distinguished by several terpenes. Are the most well-known terpenes including limonene and citral, both found in lemons, camphor, and pinene, eugenol, found in cloves, Anatol, thymol, geraniol, and menthol, all found in pine trees and cannabis, they are found in the plant's resin glands (Ceylan, 1987). There are over 100 different terpenes that have been identified in cannabis, and each strain may contain different combinations and concentrations of these compounds. Terpenes can have a variety of effects on the human body, depending on their chemical makeup (Ramos da Silva et al., 2021). For example, some terpenes may have antiinflammatory properties, while others may have anti-anxiety or anti-depressant effects. Some terpenes may also work in conjunction with cannabinoids, such as THC and CBD, to

enhance their therapeutic benefits. Additionally, terpenes may additionally play a position inside the entourage effect, that is the theory that the specific compounds in cannabis work collectively to create an extra full-size therapeutic effect than any one compound alone. In summary, terpenes are essential components of cannabis that contribute to its flavor and aroma, as well as potential therapeutic benefits (Khaleel et al., 2018). Understanding terpenes and their effects can help individuals select strains that best suit their needs and preferences.

2.2. Structure and Classification of Terpenes

Terpenes are a diverse group of organic compounds made up of isoprene units, which are five-carbon building blocks (Figure 2.1). Terpenes can be categorized into many groups based on the quantity of isoprene units they contain (Kim, 2018; Wang et al., 2018).

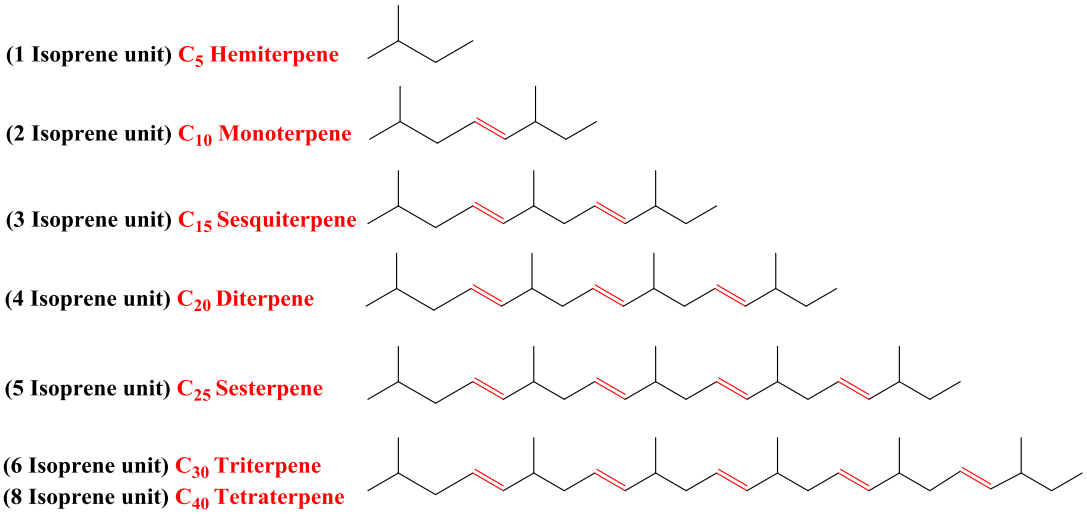


Figure 2.1. Classification of terpenes by isoprene units.

Monoterpenes: Monoterpenes are 10-carbon compounds formed by the combination of two isopentane molecules. They can be acyclic and mono or bicyclic some a typical example of monoterpene chemicals (Figure 2.2) (Kim, 2018; Mabou and Yossa, 2021). The most prevalent class of terpenes found in cannabis is citrus-smelling limonene can be found in citrus fruit oils. The oils of coniferous trees have the aromatic compounds alpha- and beta-pinene, which smell like pine. Many biological characteristics of monoterpenes include antiseptic, antibacterial, antifungal, and antiinflammatory effects and they may have neuroprotective benefits and have the potential as anticancer drugs (Croteau et al., 1988; Nikitina et al., 2017; Ramos da Silva et al., 2021).

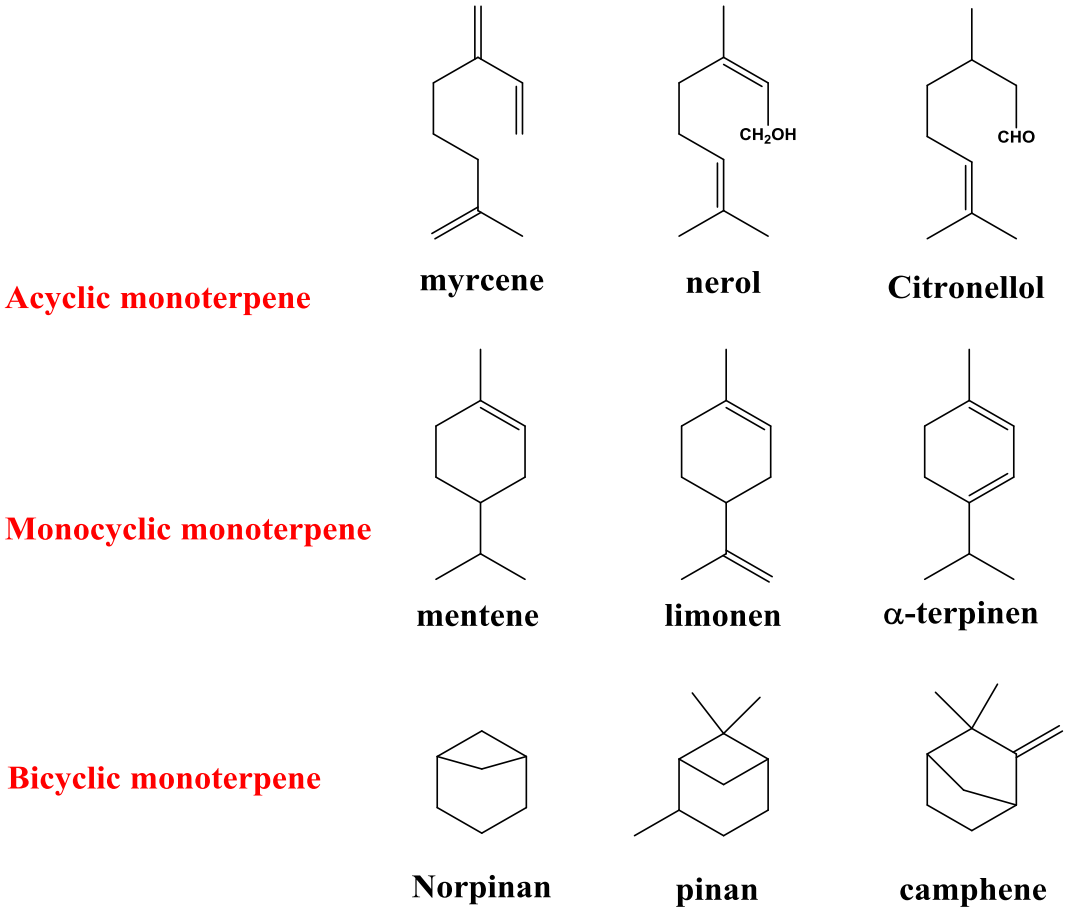


Figure 2.2. Acyclic, monocyclic, and bicyclic monoterpenes.

Sesquiterpenes: Sesquiterpenes consist of three isoprene units' molecules, beta-caryophyllene, humulene, farnesene, and bisabolol are a few examples that are frequently used. Black pepper contains beta-caryophyllene, which has a spicy, woodsy aroma. Hops contain humulene, which smells earthy and woodsy. Apples contain farnesene, which provides a green, fresh aroma. Chamomile contains the chemical bisabolol, which smells flowery and woody. Sesquiterpenes also possess a range of biological qualities, such as antibacterial, antifungal, antiseptic, and anti-inflammatory (Hossain et al., 2008; Ramos da Silva et al., 2021).

Diterpenes: These contain four isoprene gadgets, and are less commonplace than sesquiterpenes. Examples of diterpenes encompass phytol and geranylgeraniol (Ramos da Silva et al., 2021).

Sesterpenes: These contain 25 carbon atoms and five isoprene units. They are less common than other terpenes, such as monoterpenes and sesquiterpenes, but they have been found in a variety of organisms, including fungi, bacteria, and marine organisms. Sesterpenes have a diverse range of chemical structures (Mabou and Yossa, 2021).

Triterpenes: These contain six isoprene units and are less common than diterpenes. Examples of triterpenes include beta-sitosterol and lupeol (Ramos da Silva et al., 2021).

Tetraterpenes: These contain eight isoprene units and are the least common type of terpenes found in cannabis. Examples of tetraterpenes include carotenoids and chlorophyll. Terpenes can also be classified based on their chemical structure which includes acyclic, monocyclic, and bicyclic terpenes (Mabou and Yossa, 2021). Acyclic terpenes have a linear structure, while monocyclic terpenes have a single ring structure, and bicyclic terpenes have two fused ring structures overall, the structure and classification of terpenes are essential for understanding their properties, functions, and potential therapeutic benefits (Hachlafi et al., 2023; Ramos da Silva et al., 2021).

2.3. Existence of Camphene and Its Benefits

(±)-Camphene is one of a kind of monocycloterpenes (Figure 2.3). These molecular compounds of terpenes are aromatic oils that give their fragrance carrying a musky and pungent earthy scent with a piney undertone and an array of medicinal benefits, cleansers, and tastes (Quintans-Júnior et al., 2013).

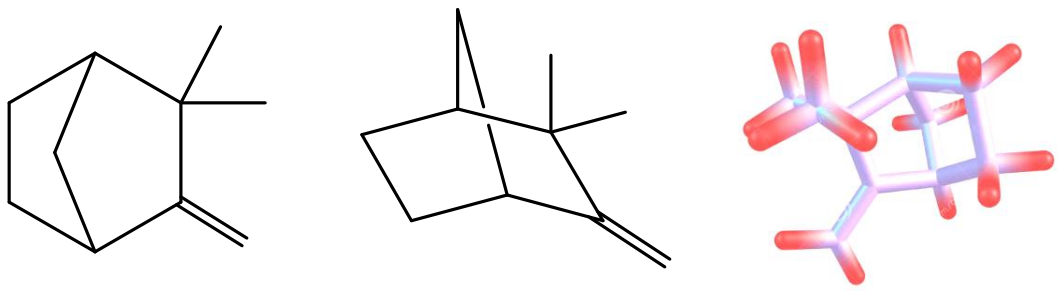


Figure 2.3. (±)-Camphene structure.

(±)-Camphene is found in nutmeg and ginger naturally, conifers, and rosemary, serving as a key component in creams, oily, and fragrances. During the 1800s, camphene was used as fuel in place of kerosene. Are explosive unique lamps are made for. Currently, scent, perfume, and camphene are industrially used chemical intermediates (Croteau et al., 1988; Banthorpe and Baxendale, 1968). Oils that include camphene include turpentine, cypress, citronella, scarlet oil, and others. It forms a small part of the plant oil, apricot, carrot, cinnamon, ginger, cumin seeds, nutmeg, cardamom, and turmeric are additional ingredients in camphene (Harborne et al., 1993). Michael Thurman in 2020 to the analysis of cannabis camphene as a

monoterpene with a bicyclic structure and a pungent odor it is used as an additive in meals and as a perfume additive (Thurman, 2020). When camphene is found in hashish, it does give the oil a slightly piney fragrance as well as an earthy or musky one. According to reports, camphene has the potential to heal skin conditions like psoriasis and eczema. Thus, (±)-camphene may be one of the more advantageous monoterpenes in hashish lotions. It may be of interest for more research due to its membrane-defensive and antithrombotic properties, so it is from time to time synthesized from more another's terpenes like pinene (Nikitina et al., 2017).

2.3.1. Camphene specifications and names

(±)-Camphene with chemical formula $C_{10}H_{16}$ and a molecular weight of 136.24 g/mol, having primary congeners D-camphene and L-camphene is referred to as (CAS No. 79-92-5) (Figure 2.4). It is a drab solid with and melting point of 50°C. (±)-Camphene does not dissolve in water, while cyclohexane, in natural solvents that include alcohol and chloroform, is effortlessly soluble. The refractive index is 1.45 and the density is 0.90 g/cm^3, the flash factor is 39°C. Its boiling factor stages from (156-160°C). Toxaphene and camphor are both called camphene. Flavorings and aromatics, such as isobornyl acetate, and organic compounds forming other chemicals, function as intermediates (Kim, 2018).

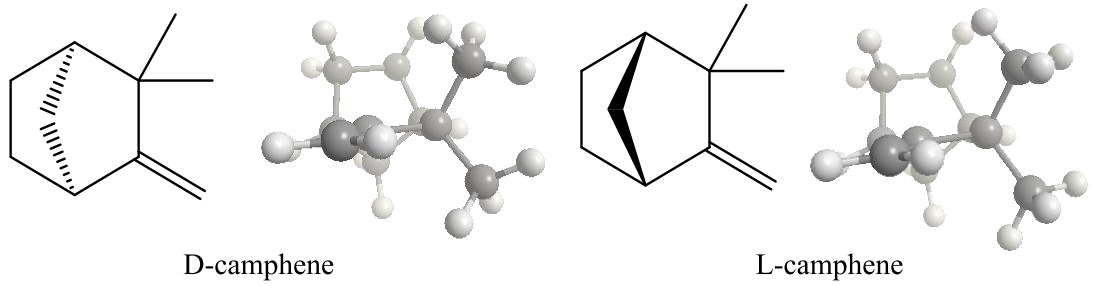


Figure 2.4. D-camphene and L-camphene structure.

Preferred IUPAC names of (±)-camphene:

3,3-Dimethyl-2-methylene-norcamphene, 2,2-Dimethyl-3-methylenebicyclo[2.2.1] heptane, 2,2-Dimethyl-3-methanylidenebicyclo[2.2.1]heptane, other names of (±)-camphene:

(1*R*)-2,2-Dimethyl-3-methylenebicyclo[2.2.1]heptane.

(1*R*,4*S*)-2,2-Dimethyl-3-methylidenebicyclo[2.2.1]heptane.

(1*R*,4*S*)-6,6-Dimethyl-5-methylidenebicyclo[2.2.1]heptane.

Sometimes known as methylenenorbornan [(1*R*,4*S*)-2,2-Dimethyl-3-methylenenorbornane]. As with organic compounds, it is traditional to use the D and

L nomenclature with camphene based on the configuration as (*S*-configuration at the α -carbon). (+)-Isomer name is [(D-Camphene), (+)-Camphene]: (1*R*,4*S*)-2,2-Dimethyl-3-methylenbicyclo[2.2.1]heptane, (1*R*)-2,2-Dimethyl-3-methylenebicyclo [2.2.1]heptane, and (−)-isomer name is [(L-Camphene), (-)-Camphene]: (1*S*,4*R*)-2,2-Dimethyl-3-methylidenebicyclo[2.2.1]heptane, (1*S*)-2,2-Dimethyl-3-ethylenebicyclo [2.2.1]heptane.

2.4. Terpenes Reactions

Terpenes are a large and diverse class of organic compounds found in many plants and animals (Quintans-Júnior et al., 2013). They are known for their characteristic aromas and flavors, and they have a variety of biological functions. Some common reactions of terpenes are:

Oxidation: Terpenes can be oxidized to form various products, such as alcohols, ketones, and aldehydes, for example, limonene, a common terpene found in citrus fruits, can be oxidized to form carvone, which is responsible for the aroma of spearmint (Hachlafi, et al., 2023).

Isomerization: Terpenes can also undergo isomerization, which involves rearranging the atoms within the molecule to form a different isomer for example, α-pinene can be isomerized to β-pinene (Wang et al., 2018).

Polymerization: Terpenes can also polymerize, or combine with other molecules of the same type, to form larger molecules. Resins and other natural compounds may result from this method (Chen et al., 2013).

Hydrogenation: Terpenes can be hydrogenated, or reacted with hydrogen gas, to form saturated hydrocarbons. This process can produce terpenes artificial versions of new properties (Christianson, 2017; Souza et al., 2019).

Reduction: Terpenes can also be reduced, which involves adding electrons to the molecule to reduce its oxidation state, for example, geraniol, a terpene found in roses, can be reduced to form the alcohol nerol (Hachlafi et al., 2023; Mabou and Yossa, 2021). Overall, terpenes are highly reactive and can undergo various chemical reactions, which can be exploited for multiple industrial and medical applications.

2.5. Wagner-Meerwein Rearrangement Mechanisms in Bicyclic Monoterpenes

The regulation of bicyclic monoterpenes has been the focus of attention for years and still is ionic addition reactions in bicyclic monoterpenes occur because of the Wagner-Meerwein rearrangement (Birladeanu, 2000). The Wagner-Meerwein rearrangement in bicyclic monoterpenes involves the migration of a substituent, such as a methyl group, from a carbon atom on the double bond to an adjacent carbon atom on the single bond (Chen et al., 2013; Smith, 1999). This process is usually accompanied by the formation of a carbocation intermediate, which can be stabilized by resonance or neighboring groups. The Wagner-Meerwein rules predict the rearrangement's outcome based on the carbocation intermediate's stability (Figure 2.5) (Adamczyk-Woźniak et al., 2021).

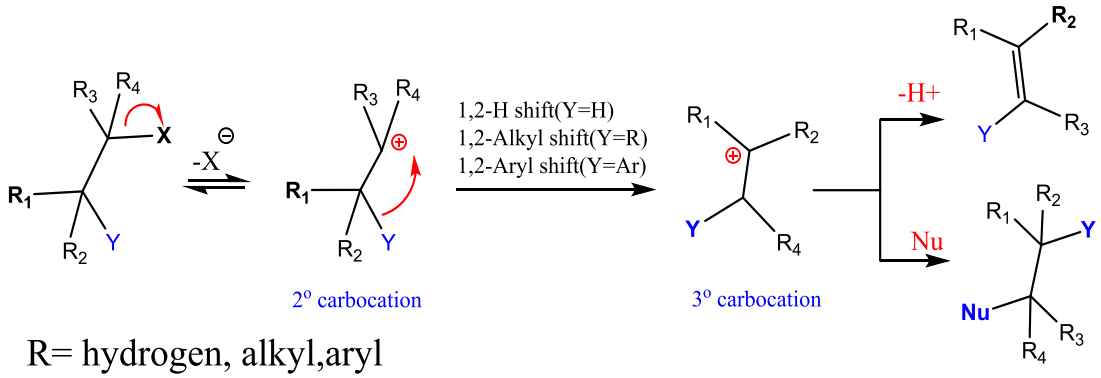


Figure 2.5. Wagner-Meerwein rearrangement mechanisms reaction.

Mechanisms of the Wagner-Meerwein rearrangement have been proposed for these products isobornyl chloride is formed because of the addition of hydrogen chloride to camphene from bicyclic monoterpenes, while bornyl chloride has been reported to form in α, β-pinene (Balkancı, 2010; Smith, 1999). This can be achieved by migrating the substituent to a more highly substituted carbon atom, or to a carbon atom that is closer to a carbonyl or other electron-withdrawing group. (±)-Camphene (1) is transformed into isobornyl chloride (4) by the addition of hydrogen chloride from bicyclic monoterpenes, and can see that with α,β-pinene (5,6) and bornyl chloride (9) are formed while chloride is formed for this mechanism has been proposed (Figure 2.6) (Titova et al., 1995).

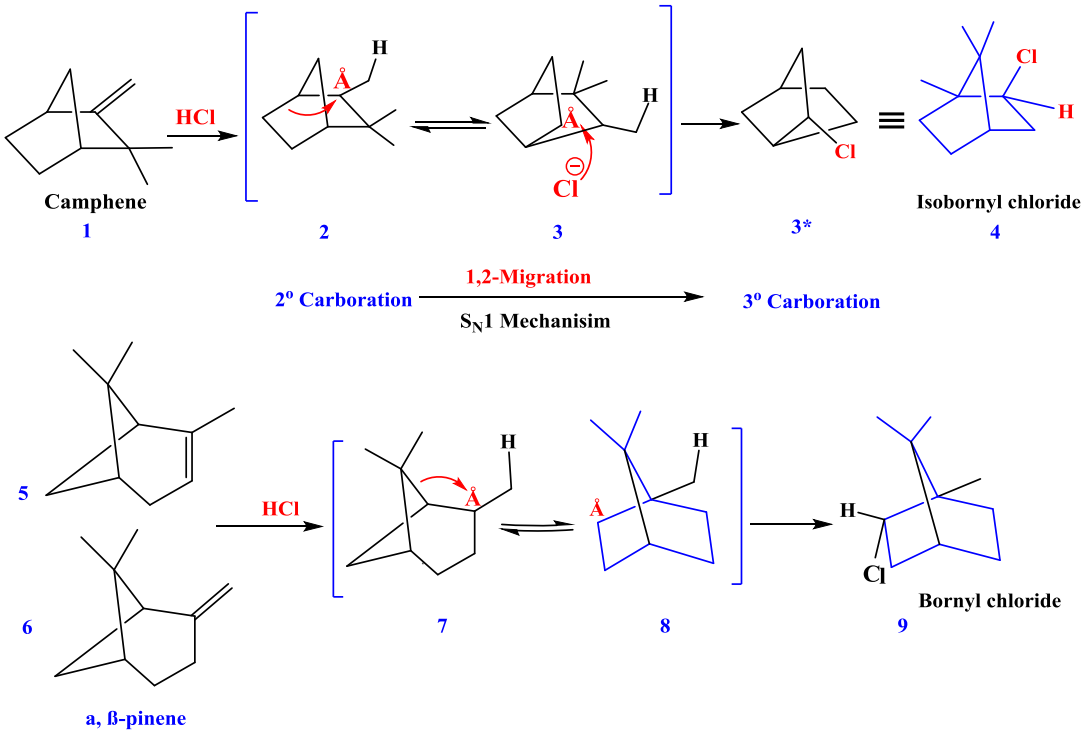


Figure 2.6. (±)-Camphene is transformed into isobornyl.

2.6. Brominated Organic Derivatives and Their Importance

The most common of these is naturally occurring bromomethane. One of the most notable applications of synthetic brominated organic compounds is the use of polybrominated diphenyl ethers (PBDEs) as fire retardants. The usage of brominated molecules as an intermediary or a coloring agent is widespread across the paint industry. Important components used in the creation of azo dyes include bromo dinitroaniline which is a supplementary item (Figure 2.7) (Tutar, 1999).

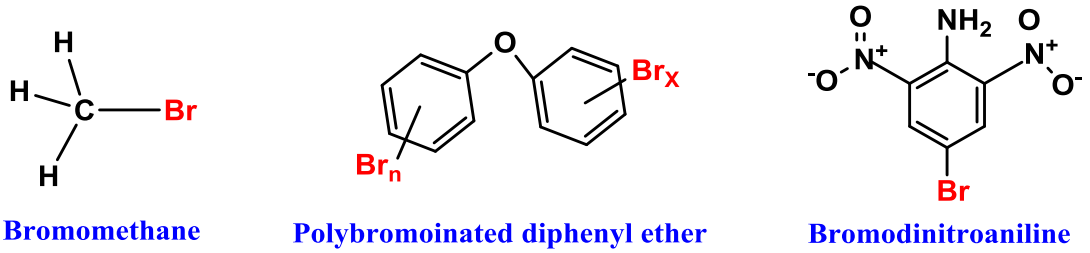


Figure 2.7. Bromomethane, polybrominated diphenyl ether, bromodinitroaniline.

Bromine has other applications as fumigants and biocides, dyes, pharmaceuticals, and designer drugs the manufacture of fire retardants is the primary industrial use of bromine. A variety of secondary bromine compounds are also found in nature, and due to their impact on the environment, these compounds have come under increased

scrutiny. Bromine compounds are relatively nonpolar like most organohalides (Tutar, 1999). Bromine is a dark red liquid at room temperature more electronegative than carbon. The carbon in the carbon-bromine bond is electrophilic, they are alkylating agents. The carbon atoms connected to bromine are very vulnerable to nucleophilic attack because the carbon-bromine bond is very weak (C-Br: 276 kJ/mol) (Balkancı, 2010; Hachlafi et al., 2023). Because of this, aryl bromides supply a fairly simple displacement reaction. A transition to practically any molecule by electrophilic displacement is possible with aromatic bromides since they also make metal-halogen exchange simple (Smith, 1999). Aryl bromides function as an intermediary and essential part in the conversion to other derivatives thanks to these characteristics. For many applications, organic bromides stand for a compromise in terms of reactivity and cost. The bromine compound family is gradually expanding, and new compounds are constantly being created for both established and emerging applications (Balkancı, 2010; Sokolova et al., 2021). The major reactions of organic bromides include reductive conjugation, debromination, nucleophilic substitution, and grignard interactions.

2.7. Bromination Reactions

Overall, bromination reactions are useful tools for introducing bromine atoms into organic molecules, and they find applications in a wide range of fields, including pharmaceuticals, materials science and chemical synthesis. There are several different types of bromination reactions, including electrophilic bromination although the electrophilic addition of bromine to olefins is a well-known and widely researched reaction, the bromination reaction and the nature of the intermediate are still controversial (Balkancı, 2010; Brown, 1997). The mechanism of bromination reactions which is one of the basic reactions of unsaturated compounds is complex (Figure 2.8).

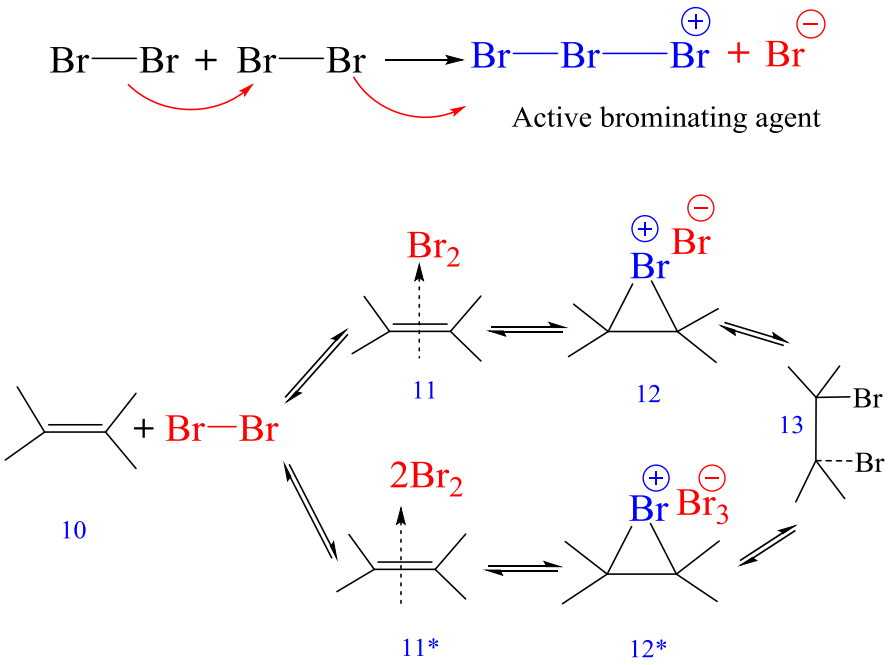


Figure 2.8. Electrophilic bromination mechanism.

As a result of the first attack of bromine in the mechanism, a bridged bromonium or bromo carbonium ion (11, 11*) is formed first (Acer, 2009). It is accepted that bromide (12) or tribromide anion (12*) is formed as a counter ion, depending on whether the solvent is protic or aprotic. These ion pairs rapidly turn into dibromide products (13) because of the ionization of the weak charge transfer complex (Demirci Gültekin, 2005). In addition to kinetic information, product stereochemistry, and effects are explained based on this basic mechanism. The formation of cyclic bromonium ions as an intermediate in the electrolytic bromination of olefins was first described by Roberts and Kimball 1937 reported that adamantanylidene (14) to adamantane (15) is brominated (Demirci Gültekin, 2005). The X-ray structure by isolating bromonium tribromide salt from the reaction definitively revealed the structure of the three-membered bromonium ion they have put (De la Mare et al., 2013). In addition, this salt is completely alternating again with Br_2 in the bromination reaction, showing that it is converted from adamantanylidene to adamantane by the same group in which the bromonium salt is formed and the intermediate is alternating shown (Figure 2.9) (Brown, 1997).

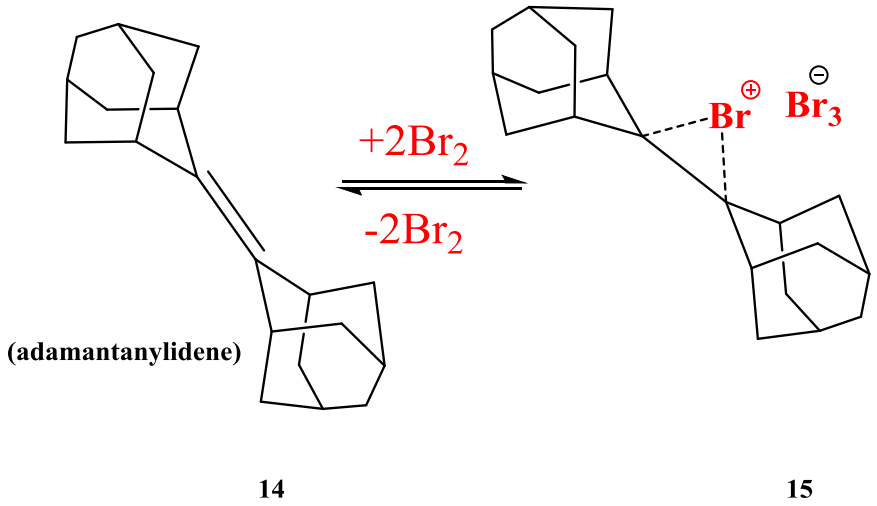


Figure 2.9. Adamantanylidene bromination reaction.

It is used as an intermediate in the bromination reactions of unconjugated olefins bromonium ion is formed. These reactive intermediates then produce antiaddiction products (De la Mare et al., 2013).

2.7.1. Bromination in bicyclic systems

The bromination of rigid alkenes in bicyclic structure is quite different from the bromination of classical alkenes (Titova et al., 1995). While bromination reactions under normal conditions run on the bromonium ion, in the bromination of such molecules mostly the bromonium ion is regulated to the non-classical carbocation. Thus, bromination proceeds via the non-classical carbocation.

2.7.1.1. Classic and nonclassical carbocation

The key difference between nonclassical and classical carbocation is that classical carbocations (16) have a carbon atom having six electrons in three chemical bonds, whereas the three-center, two-electron (18) structure of nonclassical carbocations (Figure 2.10) (Balkancı, 2010). In the transition ion (20) is formed during the rearrangement of classical and nonclassical carbocations (±)-camphene hydrochloride (19) which has a bicyclo heptane skeleton structure, into isobornyl chloride (21) (Nevell et al., 1939). In later years, Meerwein defined such products in chemical reactions as carbonium ions (Titova et al., 1995; Yılmaz et al., unpuslished). Winstein and Trifan examined the solvolysis of 2-norbornyl tosylate and defined the intermediates formed as nonclassical carbocations (Figure 2.11) (Winstein, 1961; Winstein and Trifan, 1952).

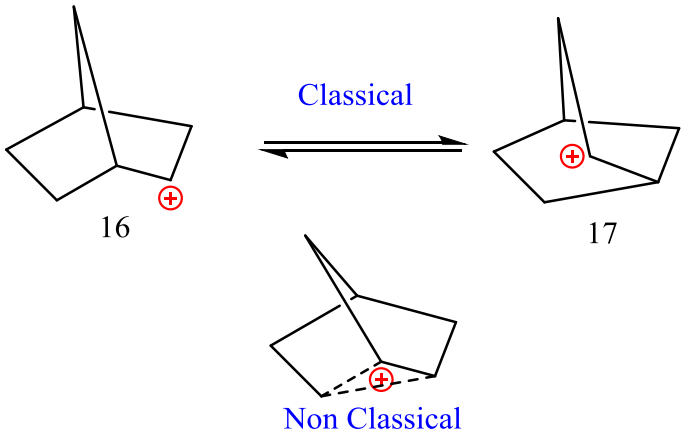


Figure 2.10. Classic and nonclassical carbocation.

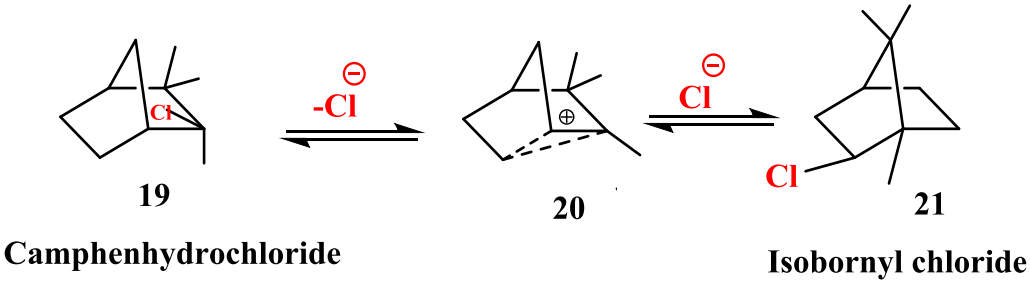


Figure 2.11. Adding chlorine to (±)-camphene.

Based on their studies, the same group suggested that the norbornyl cation, which they formulated as (22) has three resonance rings in the form of (23-26) (Figure 2.12).

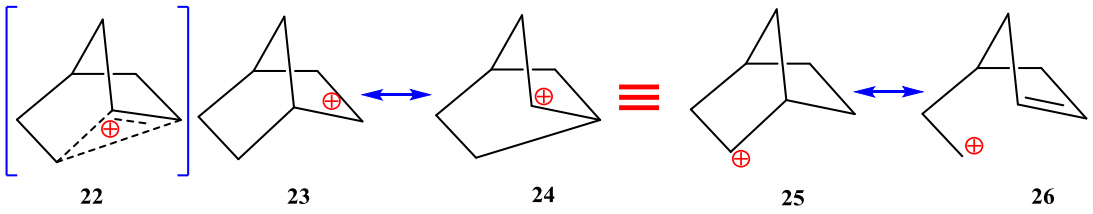
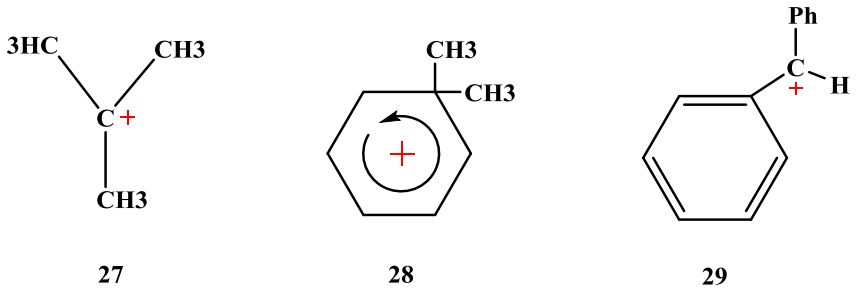


Figure 2.12. Mechanism of adding chlorine to norbornyl.

In allylic or benzylic cations, the positive charge can be distributed over different carbons. However, in such a delocalization event, molecular orbitals are formed because of π-atomic orbitals interfering with the carbocation center. Such intermediates are known as classical carbocations (Figure 2.13). Cation (27-29) is stabilized by the ($^{+}$I) effect of methyl groups and by hyperconjugation. There is no distribution of the positive charge in this molecule on the methyl carbons (Gültekin et al., 2008).



27 28 29

Figure 2.13. Classic examples of carbocations.

Nonclassical carbocations are distributed on different carbons because of intramolecular delocalization via σ-electrons instead of positive charge π-electrons. This statement is the biggest property that distinguishes nonclassical carbocations from classical carbocations. (Gültekin et al., 2008). Collins and Harding have seen products in which the labeled carbon is dispersed to unusual positions because of the 2-cyclopentenylethyltosylate (30) and acetolysis of 2-*exo*-norbornyltosylate (31) obtained results show that ionic reactions in bicyclic systems continue over nonclassical carbocations. Electrophilic addition reactions of bicyclic olefins generally proceed through nonclassical carbocations (Figure 2.14) (Collins and Harding, 1969). As a result of this, both skeletal regulation and stereochemical changes are seen. The bromination reactions of such systems have been studied in a wide range (Gültekin et al., 2008).

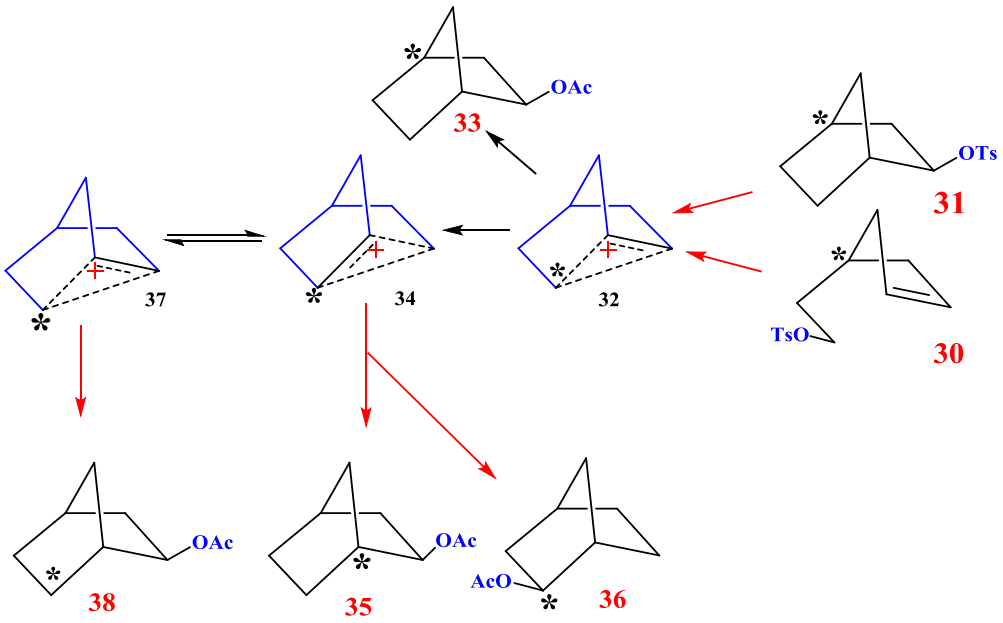


Figure 2.14. Non-classical carbocation rearrangement products.

2.8. Bromination at Low Temperatures

In the bromination of norbornadiene (39) in chlorinated solvents at low temperatures (0ºC), dibromide (42) formed as a result of the Wagner-Meerwein rearrangement and as a result of homoallylic conjugation, nortricyclanic compounds (40 and 41) were obtained (Winstein and Trifan, 1952). In this reaction, normal addition products (no formation of trans dibromide (43)) indicate that the reaction over bromonium ion cannot compete with the nonclassical carbocation (Figure 2.15).

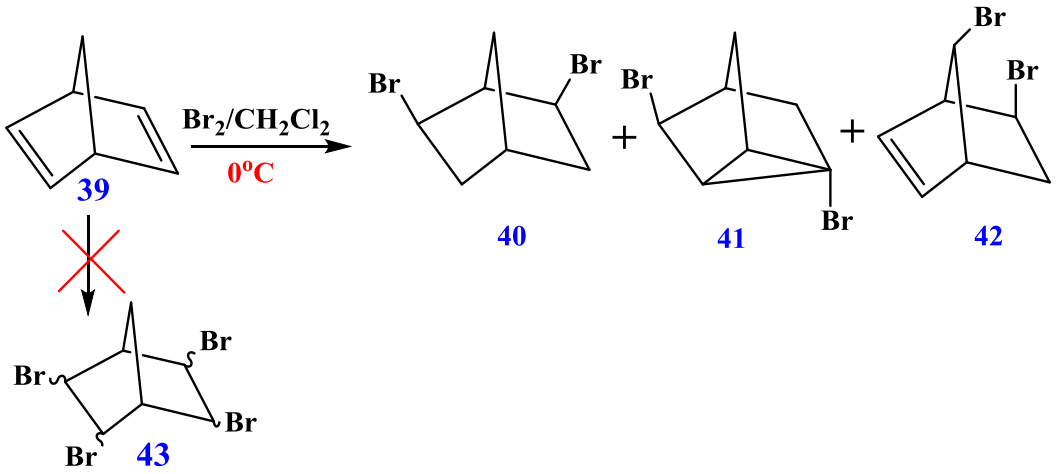


Figure 2.15. Low temperature bromination of norbornadiene.

So, the low temperature bromination of benzonorbornadiene (44). The result of the bromination reactions of benzonorbornadiene (44) at room temperature and lower temperatures is a quantitatively regulated product (46) (Figure 2.16).

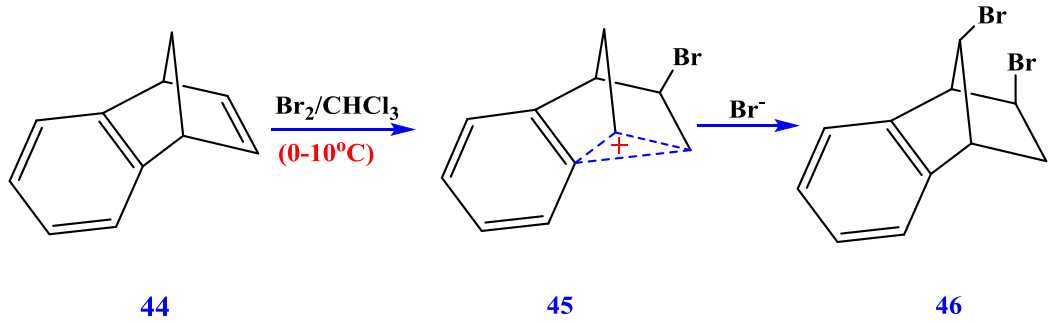


Figure 2.16. Low temperature bromination of benzonorbornadiene.

Nortricyclanic products formed in the bromination of norbornadiene were not observed in this molecule. Because the formation of such a product causes the deterioration of aromaticity (Meinwald and Wiley, 1958). In such a system, bromine can approach the double bond electrophilically in two ways. As a result of the attack

by the benzo ring *endo*-bromonium ion (45) and the attack in the direction of the methane bridge, *exo*-bromonium ion (48) is formed. There are certain factors that decide the formation of these two intermediates. The stereochemistry of electrophilic addition to double bonds in bicyclic systems depends on some effects such as steric factors, torsional effect, interference of π and σ orbitals in transition, formation of nonclassical ion, and rapid equilibrium with classical ion (Titova et al., 1995). The stereochemistry of the electrophilic addition of benzonorbornadiene (44) depends on the stabilization of the transition state nonclassical carbocations (46 and 49) by the interference of π and σ electrons (Figure 2.17) (Tutar and Balci, 2002).

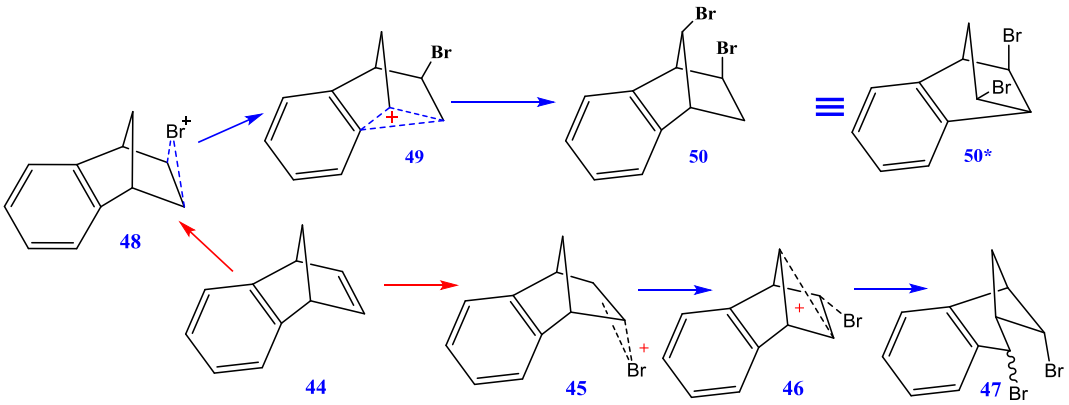


Figure 2.17. Formation mechanism of benzonorbornadiene.

When the *exo*-bromonium ion (48) is formed, this ion is stabilized by the interference of the aromatic ring π orbitals, and eventually, the bromonium ion transforms into the homobenzyl ion (49) which is more energetically stable (Balkancı, 2010). The *endo*-bromonium ion (45) formed as a result of approaching bromine from the *endo* face, on the other hand, can only be stabilized as a result of interference of σ electrons. This is less preferable. In addition, there is an aryl migration over the *exo*-bromonium ion, and the arranged product in the skeletal structure is transformed into (50, 50*). Only alkyl migration can occur via *endo*-bromonium ion. The product (47) formed because of alkyl migration is more in the tensile skeleton structure (Balkancı, 2010).

2.9. Low Temperature Bromination of (±)-Camphene

Camphene's responses to bromination have been known since the late 19th century all the research that was done either had insufficient experimental data or was unable to decide the structure analyses because there were no speculative measurements. Titova et al., in 1995 conducted significant research on the bromination of (±)-camphene three

compounds were shown from the reaction between molecular bromine and (±)-camphene (1) methylene chloride in the relevant study. Bromo-(±)-camphene which is a byproduct of addition separation is one of these products (51) (Titova et al., 1995; Yılmaz et al., unpublished). It is isolated from bromocamphene with rather poor yields (Gültekin et al., 2008). It is understood that the arranged items combine to generate (52) primary products (Figure 2.18).

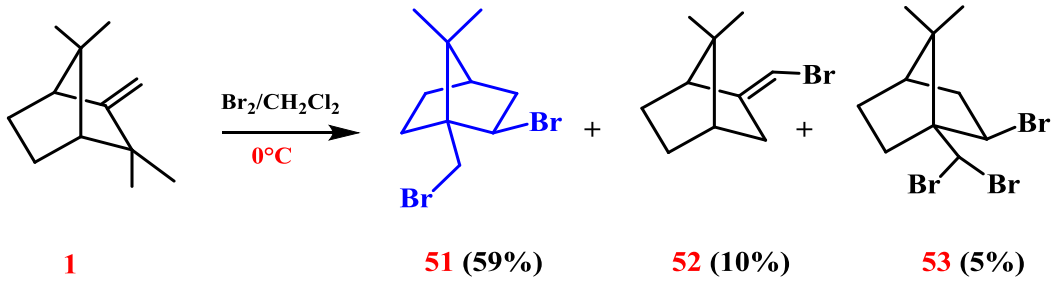


Figure 2.18. (±)-Camphene bromination.

According to the study in question, the following mechanism governs how products are formed. The Wagner-Meerwein rearrangement produces the arrangement products (52 and 53), as may be inferred from the method depicted in (Figure 2.19).

The mechanism shows how non-classical carbonation is used to carry out bromination (Gültekin et al., 2008; Horasan et al., 2003; Tutar, 1999).

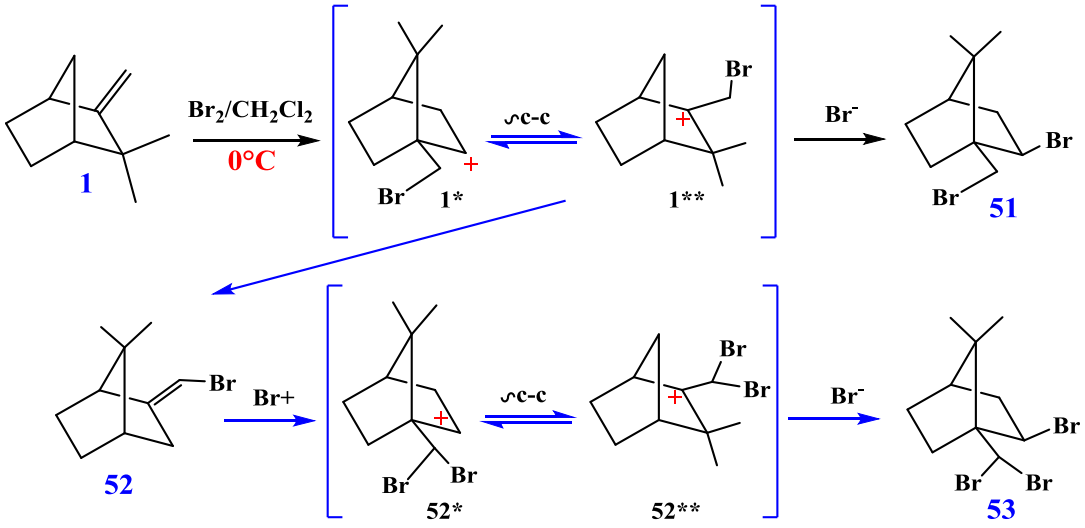


Figure 2.19. (±)-Camphene bromination mechanism.

2.9.1. Camphene reactions

In a study of (±)-camphene (1) with aqueous acetic acid in the presence of heteropolyacid, acetate, and hydroxide compounds, which are the products of regulation, were synthesized (Figure 2.20) (Souza et al., 2019).

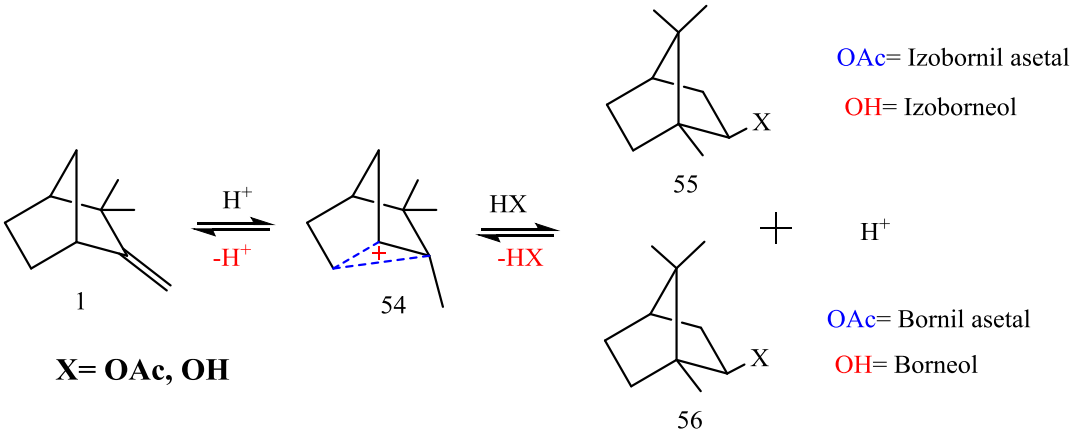


Figure 2.20. (±)-Camphene acetylation in the heteropolyacid.

Isobornyl nitrate was obtained from (1) as the main product in the study in which N_2O_4 was used as a solvent in the interaction of (±)-camphene with N_2O_4 and concentrated HNO_3 (Balkancı, 2010). In this reaction, zeolite was used as a catalyst in a concentrated HNO_3 medium. Depending on the structure of the solvent and the ratio of the zeolite product primarily intronization product (58) was obtained (Figure 2.21).

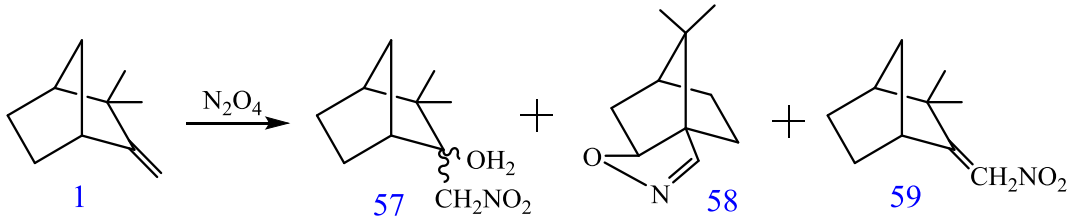


Figure 2.21. (±)-Camphene interaction with N_2O_4 and HNO_3.

In 2006 Lana obtained diisobornyl ether from camphene by using heteropolyacid (H_3PO_4) as a catalyst in their study (Figure 2.22) (Lana et al., 2006).

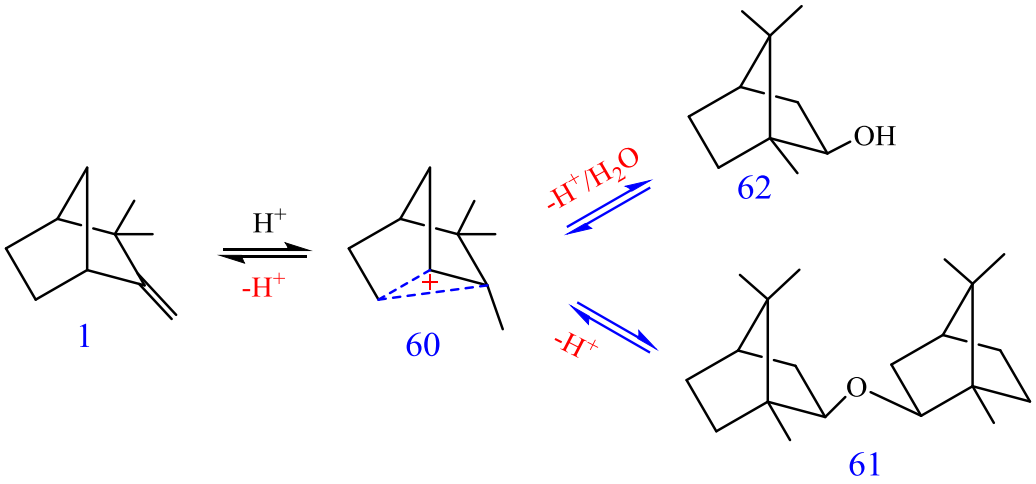


Figure 2.22. Synthesis of diisobornyl ether from camphene.

This reaction unlike what we mentioned before was not in acetic acid but in nitrobenzene and benzene sequentially (Lana et al., 2006). In most cases, isobornylether (61) was obtained as the main product in homogeneous and heterogeneous systems little amount of isoborneol (62) was observed to occur. In some cases, besides isobornylether and isoborneol, very few oligomerization products have been isolated. The formation of regulation products in almost all additional reactions of camphene has led to new searches. Several studies have been conducted for the synthesis of camphene derivatives without editing. Foca et al., in 2002 used tin and platinum compounds as catalysts in the diastereoselective hydroformylation of camphene, one of which was catalyzed by platinum-tin compounds in ($PtCl_2$-$(PPh_3)_2$/$SnCl_2$/PPh_3) where tin chloride is absent platinum complexes could not be effective in the hydro formation of camphene (Foca et al., 2002). In the presence of catalysts (±)-camphene (1) reacts with CO and H_2 to form linear aldehydes. When using $SnCl_2$ and platinum complexes of equal molarity, 50% amount of the product and 89-96% of this product is (63 or 63*) 15% exit isomer (36) is formed (Figure 2.23) (Foca et al., 2002).

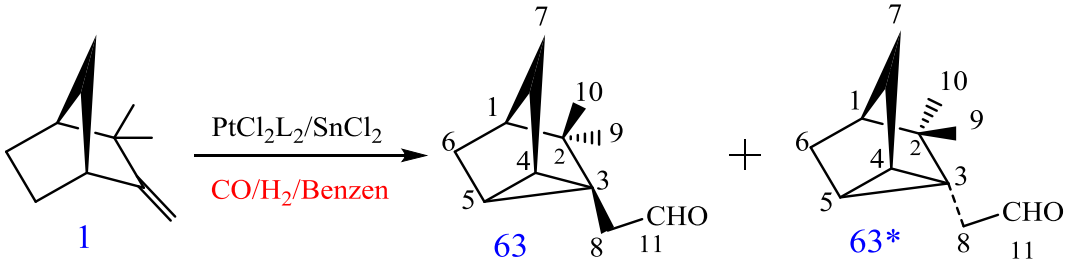


Figure 2.23. Synthesis of aldehyde from camphene.

Other significant characteristics that make (±)-camphene proper for various electrochemical reactions are, the Suzuki–Miyaura coupling reaction partly because the boronic acid compounds are low in toxicity and have good stability and simplicity of synthesis and the production of new compounds with different characteristics we explain the coupling reaction mechanism in Figure 2.24.

2.10. Suzuki-Miyaura Coupling Reactions Mechanisms

Generally can used Suzuki-Miyaura coupling reaction in organic chemical reactions about the synthesis of (C-C) bonds (Tutar et al., 1996; Gujral et al., 2012; Yılmaz et al., 2023; Yılmaz et al., unpublished). It involves using a palladium catalyst to cross-coupling between organoboronic acid and halides or triflate. Late transition metals like Pd and Ni are typically used as catalysts. The mechanism for this conventional coupling, using the "Pd(0)-Pd(II) catalytic cycle" as an example, consists of three key components (Figure 2.24) (Liu et al., 2011).

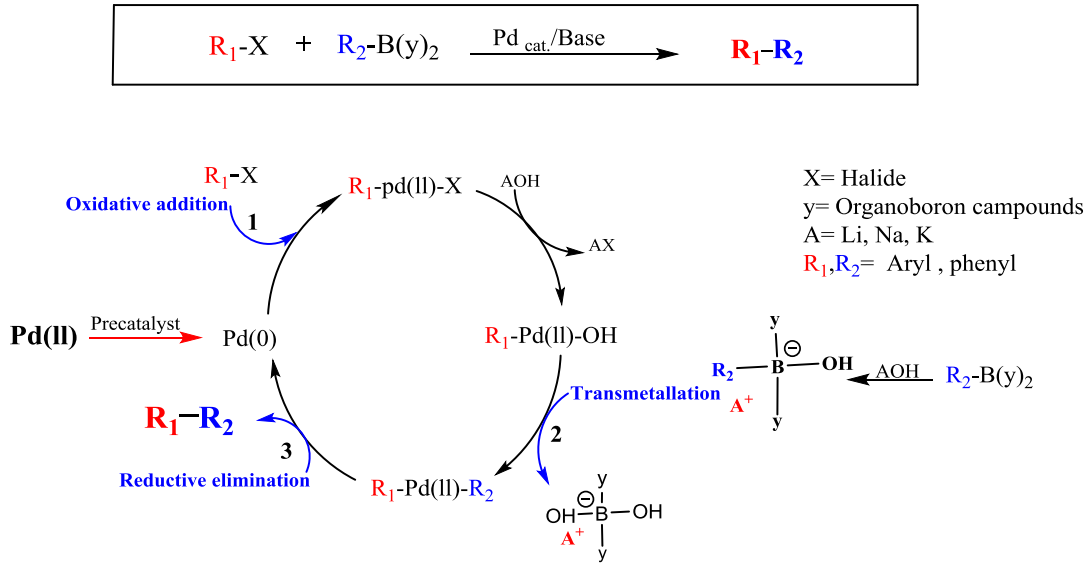


Figure 2.24. Suzuki-Miyaura coupling reactions mechanism.

1. Oxidative addition: C-hetero atom bond of electrophiles R_1-X to the Pd(0) to form the R_1-Pd(II)-X.
2. Trans metalation: Nucleophiles R_2-B(y)$_2$ with the Pd(II)-X bond to form R_1-Pd(II)-R_2.
3. Reductive elimination: The intermediate R_1-Pd(II)-R_2 to make the coupling product R_1-R_2 and finish a cycle by renewing the Pd(0) species (Figure 2.24).

So far, this model has been extensively studied and widely applied in coupling reactions to synthesize polyolefins, styrene, and substituted biphenyls which are crucial in the pharmaceutical, materials science, and electronics sectors that are both environmentally friendly and straightforward (Roglans et al., 2006). In this study we used a Pd(0) catalyst to give compounds 73-77.

2.11. Optical Rotation of Terpenes

The optical rotation of terpenes can vary depending on the specific terpene molecule, the enantiomeric form, and the experimental conditions. While optical isomers have most of the same physical and chemical characteristics, their shape matters when a difference occurs between optical rotation. A biological reaction will be aided by one optical isomer, whereas the other will either have no effect or a different effect. Some examples of terpenes and optical rotations when measured at 20°C using sodium D-line light (wavelength of 589 nm) and a standard concentration of (1 g/10 mL) in ethanol (Figure 2.25) (Wiberg et al., 2004).

α-Pinene: This is a common terpene found in the essential oils of pine trees and many other plants. It exists as a mixture of two enantiomers: (+)-α-pinene (64) and

(-)-α-pinene (65). The specific rotation values for (+)-α-pinene and (-)-α-pinene are around +17.5° and -17.5°, respectively (Wiberg et al., 2004).

β-Pinene: This is another terpene found in various plant essential oils, including those of pine trees. It also exists as a mixture of two enantiomers: (+)-β-pinene (66) and (-)-β-pinene (67). The specific rotation values for (+)-β-pinene and (-)-β-pinene are approximately +39.0° and -39.0°, respectively (Wiberg et al., 2004).

(±)-Camphene: This is a terpene commonly found in essential oils such as fir oil, artemisia annua, neroli, camphor, lavender, acorus, and turmeric. It exists as a mixture of two enantiomers: (+)-camphene (68) and (-)-camphene (69). The specific rotation values for (+)-camphene and (-)-camphene are around +104°, and -85° respectively (Quintans-Júnior et al., 2013; Mort and Autschbach, 2007; Wiberg et al., 2004).

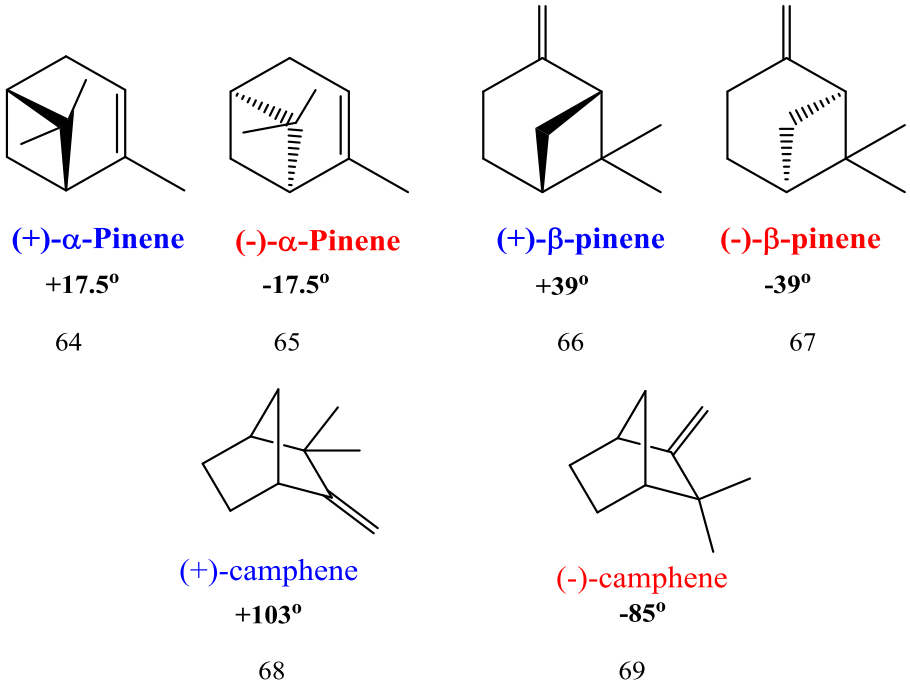


Figure 2.25. Optical rotation of terpenes.

2.12. Biological Activities of Terpenes

Over the past years, increased terpenes studies have been published on the biological properties of terpens as they have been used in traditional medicine for millennia. (Wang et al., 2018). In recent years, biological properties have been extensively demonstrated to prove the mechanism of action of essential oils for different terpenes the most important of these characteristics (Martinez et al., 2012; Smith, 1999).

1. Antioxidants (Adorjan and Buchbauer, 2010; Chukicheva et al., 2010).
2. Displays antitumor activity in vivo anticancer (Machado et al., 2022).
3. Inhibit inflammatory and neuropathic (Smith, 1999).
4. Skin penetration-enhancing activity (Quintans-Júnior et al., 2013).
5. Cardiovascular and antihypertensive effects (Ramos da Silva et al., 2021; Vallianou et al., 2011).
6. Anticonvulsant and sedative activity (Quintans-Júnior et al., 2013).
7. Antimicrobial agent and antibacterial agent and effect (De Freitas et al., 2020; Hachlafi et al., 2023; Rasoul et al., 2012).
8. Antifungal agent (Hossain et al., 2008).
9. Antituberculosis agent (Ramos da Silva et al., 2021; Souza et al., 2019).
10. Insecticidal activity (Prates et al., 1998).

11. Diminished muscle dysfunctions, such as reduced muscle mass and glucose uptake (Baek et al., 2020).
12. Reduces plasma cholesterol and triglycerides (Smith, 1999).
13. Antiviral activity against several types of viruses (Sokolova et al., 2021).

2.13. Importance and Scope of The Study

Terpenes molecular compounds are also known to have a range of medicinal benefits. Also found naturally in conifers, ginger, nutmeg, and rosemary camphene is used as a main ingredient in many topical creams, perfumes and oils, it is commonly used in creams, ointments, and lotions, and it can treat skin conditions camphene can have antifungal and antibacterial efficacy (Quintans-Júnior et al., 2013).

Since the compound (-)-camphene (69) pure is very expensive on the market and difficult to extract, it was prepared from its analogs and in feasible ways that are useful for preparing derivatives that are difficult to obtain, thus obtaining different treatments and research for this compound. In this study, all (-)-camphene-derived compounds 73-77 that were prepared were screened for the presence of the assigned biological activity. Therefore, the idea of this study is to discover new strategies for preparing compounds that are difficult to separate and prepare, as well as being expensive in the markets, and to prepare them to use the simplest means and in quantities satisfactory to industries, and such antibacterial materials. For future projects that reduce the risk of spreading the disease. Within the scope of this study, it was aimed to synthesize (-)-camphene derivatives that were starting from (-)-camphene (70) by using Suzuki-Miyaura coupling reactions and by linking different phenylboronic acids (phenyl, 4-methoxyphenyl, 4-thiomethylphenyl, 4-ethylphenyl, 4-trifluoromethoxyphenyl) with palladium as a catalyst in the presence of nitrogen gas and the base medium. In particular, the Suzuki-Miyaura reactions have been widely developed and used in the synthesis of other compounds, it has the added advantage of being easy to prepare in large quantities, and the reactions occur at a high temperature and are usually rapid. Reactions are readily monitored as they come to an end when the nitrogen is no longer released, N_2 gas is an excellent leaving group as it doesn't affect the reaction mixture; hence, a compound's

In this study, we have a levorotatory compound that took their optical rotations when measured at (23°C) using sodium D-line light (wavelength of 589 nm). Accordingly,

the findings demonstrated that 47 and 72 were capable of inducing an anticancer effect without affecting normal cells. On the other hand, when considering all treated bone cancer cell lines, the 76 and 72 molecules with strong anticancer effects are the most convenient ones for further preclinical studies because they exhibit low cytotoxicity towards normal chondrocyte HC cells. When evaluating 71, 72, and 76 molecules together with MG63 bone cancer cells, it was expressed that these molecules exhibited strong antiproliferative effects When considering Saos2 cancer cells, these molecules in the range of TGI values exhibited similar or better anticancer effects compared to the control medication, 5FU. We saw the anticancer activity exhibited by subjected molecules except for compounds 73 and 77 was found to be at a similar or higher level than the standard anticancer medication 5FU in SW1353 cancer cells. In contrast to the positive control group, 5FU, it was compound 74 very toxic against HC-normal chondrocyte cells, despite being found to be more affected by bone cancer cells. This situation limits the use of the 74 agents. If these 71 and 74 molecules' toxic effects against lung and chondrocyte normal cells, respectively, are reduced by chemical modification, they may be a good option for cancer treatment. Compound 72 was more cancer-specific for forms of bone and lung cancer, and that make it most of convenient one for further preclinical studies. The MTT and LDH test results, which show the therapeutic and cytotoxic features, respectively, were evaluated together. It was vigorously expressed that compounds 72, 74, and 76 show the most optimal cytotoxic effect against cancer and antiproliferative and normal cells compared to the positive control, 5FU. Overall, the (-)-camphene substances have the ability to start an apoptotic cascade in cells and result in the development of apoptotic DNA fragmentation. The (-)-camphene molecules are partly responsible for their anticancer activities when we did the Migration test results of 71, 75, 76, and 77 molecules and The changes that occurred in the cell morphology by applying TGI doses of (-)-camphene molecules indicate that the effect mechanism of the (-)-camphene, including apoptosis, is in line with the MTT, DNA degradation, and cell migration test results of the (-)-camphene molecules. On the other hand, these molecules lack antimicrobial properties against all bacteria and fungi. Therefore (-)-camphene compound may be a good option for cancer treatment in the future.

3. MATERIALS AND METHODS

3.1. Material

3.1.1. Solvents and chemicals

References most of the chemicals and solvents used in the tests are bought for the research, dryers, and chemical reagents from the Merck, Fluka, Aldrich, Sigma-Aldrich, Tekkim and Alfa Aesar brands are used. Technical (domestic) solvents are proper for column chromatography and washing when used after distillation over dryers in synthesis and very pure solvents are obtained by import.

Chemicals: Bromine, (-)-camphene technological caliber, 75% (90% as camphene and fenchene), (phenyl, 4-ethylphenyl, 4-thiomethylphenyl, 4-trifluoromethoxyphenyl, 4-methoxyphenyl) boronic acid, palladium(0), potassium carbonate, toluene, aniline, hydrochloric acid, and N_2 gas.

Solvents: Dichloromethane, chloroform, hexane, benzene, diethyl ether, and ethanol.

Desiccants: Calcium chloride and sodium sulfate were used.

3.1.2. Purification

The purification processes of all solvents used in the experiment were conducted by modern methods as stated in the literature (Perrin et al., 1988).

Chloroform: Chloroform brought from abroad is used directly in reactions and crystallization processes. The chloroform used in the column process was utilized from locally supplied chloroform.

Dimethyl chloride: Dimethyl chloride is used in reactions and crystallization processes. In the column and extraction processes, technical dimethyl chloride was used.

Acetone: Acetone, which is generally used in the cleaning processes of laboratory materials, is technically supplied.

Diethyl ether: While absolute pure ether is used in crystallizations, indigenous in extraction processes is used.

Hexane: In chromatographic column operations, technical hexane was used after being distilled by fractional distillation method via $CaCl_2$. Absolute hexane was used directly in the crystallization processes.

Benzene: In chromatographic column operations, technical benzene was used. Protective steps have been implemented to avoid skin contact with substances that are known to cause cancer.

3.1.3. Chromatographic analysis

In chromatographic methods in separation and purification procedures, classical column chromatography has often been used silica gel 60 (0.063-0.200 mm) as filler and (230-400 mesh ASTM) from Merck branded. Hexane was used as the eluting phase in column chromatography.

3.1.4. Spectroscopic methods

The ^{1}H NMR and ^{13}C NMR measurements required for the structure analysis of the compounds obtained in pure form at the end of our studies were taken using Varian Mercury 300 MHz NMR spectroscopy in our department. Infrared spectra were recorded from Spectrum TWO FT-IR spectroscopy in our department.

3.1.5. Rotary evaporator

Heidolph brand 4003-G3 vertical type to remove solvents in the reaction medium in a low vacuum evaporator was used.

3.1.6. Optical rotation

Measurements required for optical rotation by P8000 (A. KRÜSS Optronic, Germany) using cell a 0.5 dm, all the [α] values are units of deg cm^2 g^{-1}, and concentration units are given in g/100 mL.

3.1.7. Precision balance weighing operations.

Made with Precise branded, 220 g capacity, 0.0001 precision balance.

3.1.8. The biological effect

All the materials, tools, and cells for the biological activities of our compounds were done at the Yozgat Bozok University, Faculty of Medicine, Basic Medical Sciences.

3.2. Method

3.2.1. Bromination reactions

This is carried out at low temperatures bromination (0°C) created with an ice-water bath, bromine solution prepared in the required proportion in the pressure-compensated dropping funnel was added dropwise. Excess bromine and solvent were removed under a low vacuum. The substance is purified and crystallized in silica gel columns (Figure 3.1).

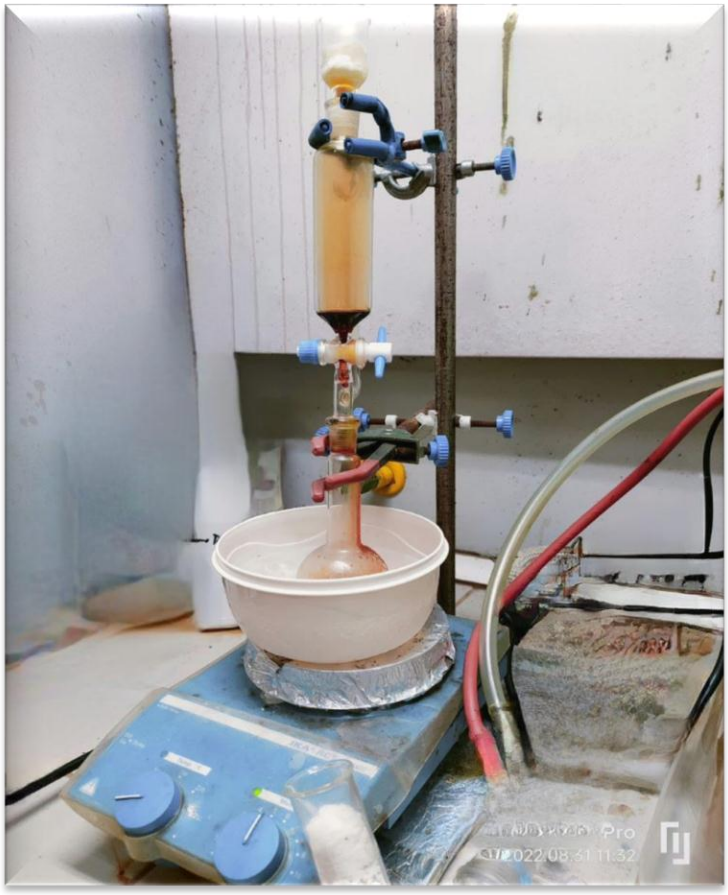


Figure 3.1. Bromination reactions of (-)-camphene.

3.2.2. Dibromo-(-)-camphene reaction

Dibromo-(-)-camphene and aniline in a reaction flask. The reaction flask is covered with thermal cotton fibers to avoid heat loss and exposure to light. Stirring of the reaction solution was permitted on the hotplate for 2h reflux. It is extracted in an organic solvent. After drying the separated organic phase. At a rotating evaporator, the solvent was eliminated. The mixture of products in the silica gel column has been divided (Figure 3.2).

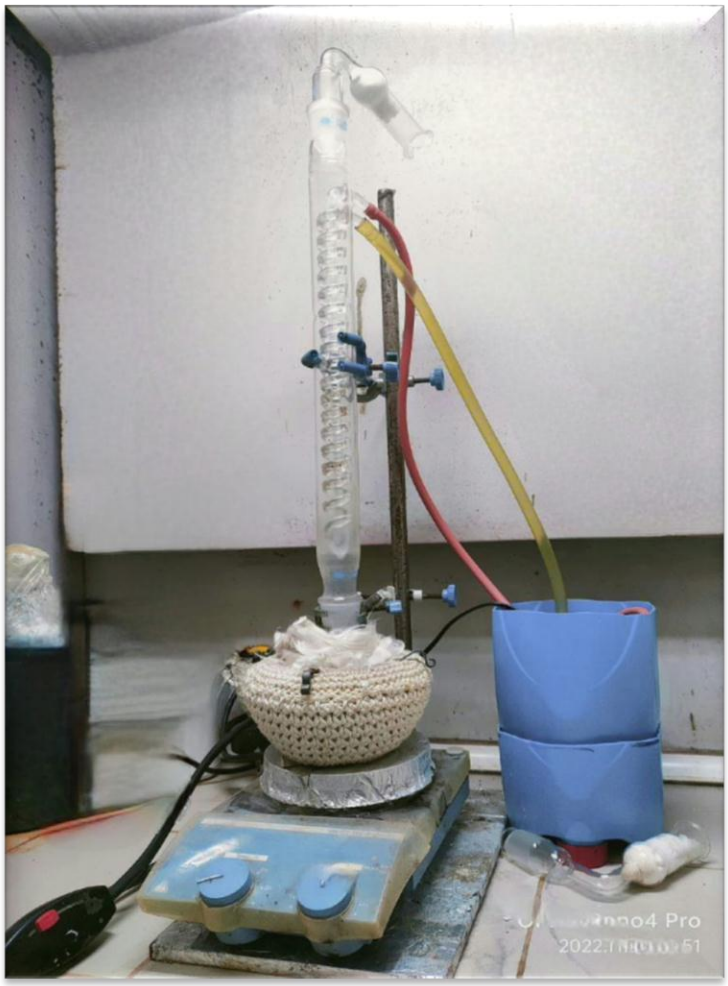


Figure 3.2. Dibromo-(-)-camphene reaction.

3.2.3. (-)-Camphene derivatives reaction

Monobromo-(-)-camphene, phenylboronic acid, potassium carbonate, $Pd(PPh_3)_4$ and toluene, ethanol and water, as a solvents into the three neck reaction flask influenced reaction balloon for 17h reflux with N_2 gas the reaction at 110ºC was stirred (Figure 3.3). The supernatant was extracted in an organic solvent and after drying the separated organic phase, the solvent was removed from the rotary evaporator. The product mix in the silica gel column has been segregated. These steps by step were repeated by using the boronic acid compounds (4-thiomethylphenyl, 4-ethylphenyl, 4-methoxyphenyl, 4-trifluoromethoxyphenyl).

Figure 3.3. Derivatives reaction.

3.2.4. Optical rotation

In a standard volumetric vial prepared for all synthesized compounds 1 g/10 mL in dichloromethane, you apply the equation to find values of specific rotation when measured at 20°C using sodium D-line light (wavelength of 589 nm).

$$[\alpha]_{\lambda}^{T} = \frac{\alpha}{C.l} \times 100$$

$[\alpha]$ = Specific rotation
T = Temperature
λ = Wavelength
α = Optical rotation
C = Concentration
l = Optical pathlength

3.2.5. Biological activities

3.2.5.1. Cancer cell culture and cell lines

In this study we used lung cancer cell lines [A549 (ATCC, CCL-185), Calu1 (ATCC, HTB-54), and H1650 (ATCC, CRL-5883)] and bone cancer cell lines [SW1353 (ATCC, HTB-94), MG63 (ATCC, CRL-1427), and Saos2 (ATCC, HTB-85)]. Beas2B (RRID, CVCL-0168) normal lung cell line, and HC (Sigma Aldrich, 402-05A) normal chondrocytes cell line. We prepared cells under sterile conditions in a laminar cabinet. As soon as the cells were confluent, they were incubated at 37ºC with 5% CO_2 in supplemented DMEM. or RPMI 1640 medium containing FBS 10% and PenStrep 2% solution. With 10000 cells per well were seeded measuring plates. After 16h approximately of pre-incubation, test molecules were added and measurements were performed after 24h of incubation.

3.2.5.2. Measurement of cell proliferation and determination of NCI-60 survival parameters

MTT test was used to measure the effects of the synthesized test 73-77 on cell proliferation and NCI-60 survival parameter values (Mosmann, 1983). After incubation, this test protocol was applied of test substances and cancer cell lines for 24h. The results were as %cell inhibition and the optical density of the solvent (DMSO) treated cells it was assumed 100%. The MTT method used on cells of increasing concentrations of each test substance [1.96, 3.91, 7.81, 15.63, 31.25, 62.5, and 125.0 μg/mL] based on a specified range of values NCI-60 survival parameters of test substances. By using a logarithmic function on the logarithmic curve prepared from the absorbance values obtained it was analyzed after the following formulas were used for the measurement of NCI-60 survival parameters (GI_{50}, LC_{50} and TGI); Cell proliferation: [(Ti-Tz) / (C-Tz)] x 100 if the [Ti>/=Tz] (cytocytic effect) or [(Ti-Tz) / Tz] x 100 if the [Ti < Tz] (cytocidal or cytotoxic effect), (Tz; zero point, C; control growth, Ti; inhibition by test substance). TGI: Concentration value that reduces growth by 100% (Ti = Tz), GI_{50}: Concentration value that reduces growth by 50% ([(Ti-Tz) / (C-Tz)] x 100 = 50), LC_{50}: concentration value that by 50% kills cells in the medium ([(Ti-Tz) / Tz] x 100 = -50).

3.2.5.3. Cytotoxicity test of (-)-camphene derivatives

By the LDH method was determined the compounds 73-77 to be tested if cytotoxic or cytostatic (Decker and Lohmann-Matthes, 1988). Increase in LDH in the culture

supernatant an increase in the amount of cells that die during the incubation period, depending on the substance tested, will result. Lactate dehydrogenase (LDH) is a stable cytoplasmic enzyme that is found in most cells, for this reason the LDH cell cytotoxicity kit was used according to the manufacturer's procedure. So the change in the amount of formazan formed as a result of LDH enzyme activity was measured and evaluated according to the formula below; %Cytotoxicity = [(Substance Absorbance - Low Control) / (High Control - Low Control) x 100].

3.2.5.4. DNA fragmentation by agarose gel electrophoresis for (-)-camphene derivatives

By using a DNA laddering assay in accordance with the standard method DNA laddering activity of the compounds was evaluated (Gong et al., 1994). Briefly, 7.5 x 10^5 cells were placed into 25 cm^2 culture flasks and treated with TGI concentrations of the compound 73-77 at 37°C with 5%CO_2 overnight. Initially, a precipitate containing DNA was taken out of the digest using a 50 µL phosphate-citrate buffer and centrifuged at 1500 x g for 5 minutes, and then 40 µL of supernatant was transferred to a microcentrifuge tube. Tween20 (5 µL) solution (0.25% in ddH_2O) and RNase A (5 µL) solution were added to the supernatant and then incubated for 30 min at 37°C. Next, (5 µL) proteinase K was added to each tube and re-incubated for 5 min at 37°C. Finally, the DNA-containing precipitate of the microcentrifuge tube was added 5 µL of loading buffer and loaded to 1.5 %agarose gel containing 0.5 µg/mL ethidium bromide and electrophoresed for 40 min at 200 mA. After electrophoresis, by UVP gel imaging system DNA fragmentation on gels was imaged.

3.2.5.5. Migration assay of (-)-camphene derivatives

A wound-healing assay was used to determine the effect of the compound 73-77 on the migration of cells. It is a method based on measuring the proliferation ability of cells in the medium containing the test substance. A two-compartment silicon well (µ-Dish) was used for this test. There is a distance of 500 µm between the two chambers of this silicon well. Cells are loaded at 35000 cells/70µL in each chamber of the two-compartment silicon well and incubated for 24 hours. After incubation, the silicon well is removed by holding it from its corner with the help of forceps, and the old medium is discarded. Then, approximately 2 mL of fresh medium is added and test molecules are placed at the GI_{50} dose. From this moment, the specimens begin to be photographed. Photographs were taken on µ-Dish, in which the test substances were

put every 24 hours, until the gap of 500 μm in the μ-Dish, which was used as a control, was filled.

3.2.5.6. Antibacterial and antifungal activity measurement with microdilution assay for (-)-camphene derivatives

In this project, *E. faecalis* **VRE** ATCC 19433, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *S. aureus* **MSSA** ATCC 29213, *S. aureus* **MRSA** ATCC 46300, *E. coli* ATCC 25922, *P. aeruginosa* **AGME** ATCC 27853, *S. gordonii* (NCTC 7870), *A. actinomycetemcomitans* (ATCC 33384), and *C. albicans* (ATCC 10231) were used. By micro-well dilution method MIC values of the compound 73-77 against bacterial strains were determined. To determine the minimal inhibitory concentration (MIC) values, inocula of bacteria were prepared using 12h Mueller-Hinton broth cultures, and to 0.5 McFarland standard turbidity suspensions were adjusted. The *C. albicans* (ATCC 1023) strain was retained on solid Sabouraud dextrose agar at 35^0 C in an incubator, 24h later, fungal inocula were prepared using RPMI-2% glucose broth and were counted using a Neubauer chamber and trypan blue stain to obtain a final concentration of $1.5x10^6$ cells/mL. Used only to count live *C. albicans* (the trypan blue counting method with the Neubauer chamber), each substance dissolved in (DMSO) and serial twofold dilutions were made in a concentration range in microplate wells containing nutrient broth from 4 to 512 μg/mL. Determined visually the growth after incubation of microorganisms at 35°C for 24h, the minimum level of focus at which there was no visible growth (turbidity) was taken as the MIC.

In this project, *E. faecalis* **VRE** ATCC 19433, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *S. aureus* **MSSA** ATCC 29213, *S. aureus* **MRSA** ATCC 46300, E. *coli* ATCC 25922, *P. aeruginosa* **AGME** ATCC 27853, *S. gordonii* (NCTC 7870), *A. actinomycetemcomitans* (ATCC 33384), and *C. albicans* (ATCC 10231) were used. By the basis of a micro-well dilution method MIC values of the compound 73-77 against bacterial strains were determined, to determine the minimal inhibitory concentration (MIC) values, inocula of bacteria were prepared using 12h Mueller-Hinton broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. The *C. albicans* (ATCC 1023) strain was retained at 35^0 C on solid Sabouraud dextrose agar in an incubator, 24h later, fungal inocula were prepared using RPMI-2% glucose broth and were counted using a Neubauer chamber and trypan blue stain to obtain a final of $1.5x10^6$ cells/mL. To count live *C. albicans* was only used the

trypan blue counting method with the Neubauer chamber. Each substance dissolved in (DMSO) and serial two-fold dilutions were made in a concentration range in microplate wells containing nutrient broth from 4 to 512 μg/mL. Growth of microorganisms was determined visually at 35°C after incubation for 24h. The lowest concentration was no visible growth (turbidity) was taken as the MIC.

4. EXPERIMENTAL AND RESULTS

4.1. Low Temperature Bromination of (-)-Camphene

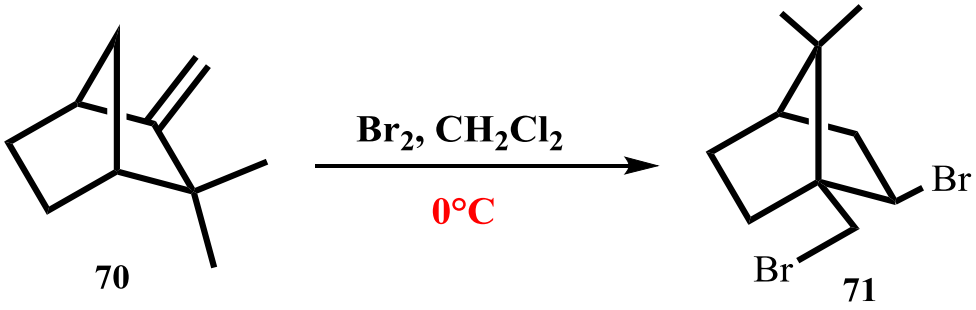


Figure 4.1. Low temperature bromination of (-)-camphene.

In a single neck flask of 4 g, (90% as (-)-camphene and fenchene) technical grade,75% (70) was dissolved in (20 mL) from CH_2Cl_2 magnetically stirred in the ice bowl. Br_2 in 20 mL CH_2Cl_2 (32.3 mmol) the solution was added dropwise over 15 minutes. The reaction medium turned brown (Figure 4.1). After stopping the dripping process, the mixing continues until it became at room temperature. 60 mL water with diethyl ether (2 x 60 mL) used to separation the oily layer and, dried by Na_2SO_4, and evaporatin of the solvent. By silica gel column chromatography using hexane the crude was purificated to gave (7.6 g, 91%) as a colorless liquid, and after 7-15 days in the refrigerator were obtained crystals (71). A supernatant was indicated by ^{1}H NMR analysis (Figure 4.2). The fact that all protons are in the aliphatic region in the ^{1}H NMR spectrum of 2,10-dibromobornan indicates that the compound is a fully saturated hydrocarbon. In the lowest part of the spectrum (4.2 ppm), the doublet of the doublet belongs to the proton on carbon 2 to which the bromine is attached. Signals in the entire aliphatic region in the ^{13}C NMR spectrum of the (71) are associated with the structure in harmony. The spectra and melting point of the compound agree with the literature were compared it was observed that all values were compatible (Figure 4.3).

^{1}H NMR ($CDCl_3$, 300 MHz, ppm) δ_H 4.33 (dd, $J_{2,3a}$ = 4.81 Hz, $J_{2,3b}$ = 4.50, 1H), 3.79 (d, J_{CH2} = 9.93 Hz, 1H, H_{CH2Br}), 3.47 (d, J_{CH2} = 9.9 Hz, 1H, H_{CH2Br}), 2.0-1.9 (m, H,

H_{3ex}, and H_{3en}), 1.85-1.80 (m, 2H, H_{6ex} and H_{6en},); 1.60-1.58 (m, 2H, H_{5ex}, and H_{5en}), 1.5 (m, H, H_{4ex}), 1.22 (s, $3H_{8CH3}$), 0.94 (s, $3H_{9CH3}$).

^{13}C NMR ($CDCl_3$, 75 MHz, ppm) δ_C 56.9, 53.4, 49.7, 48.5, 42.3, 37.5, 34.6, 26.5.

FTIR (cm^{-1}) ν = 2958 ($-CH_2$), 2885 (-CH), 1458 ($-CH_3$), 1388 ($-CH_2$), 1227 (C-Br).

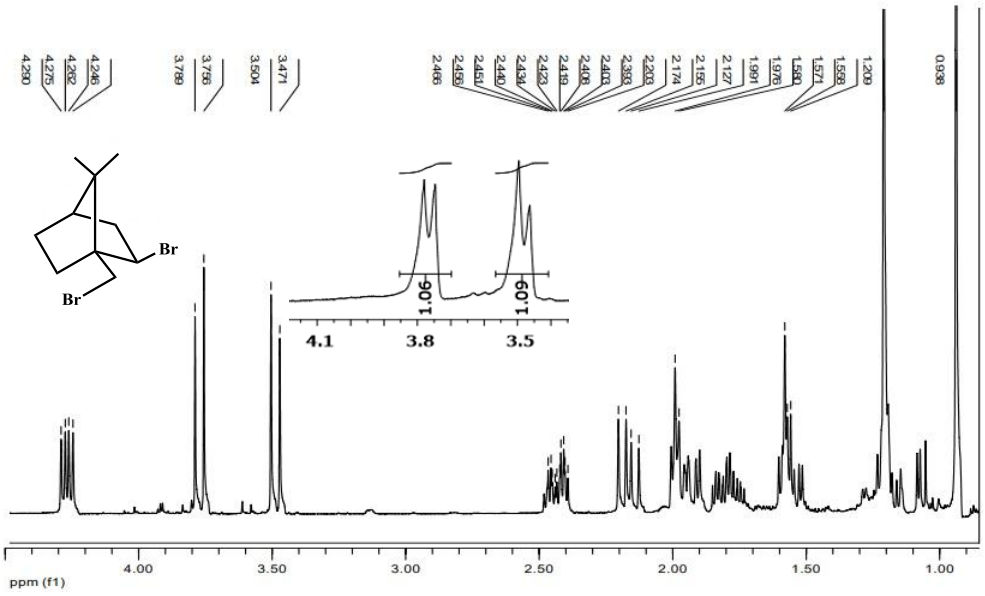


Figure 4.2. ^{1}H NMR of 2,10-dibromo-(-)-camphene.

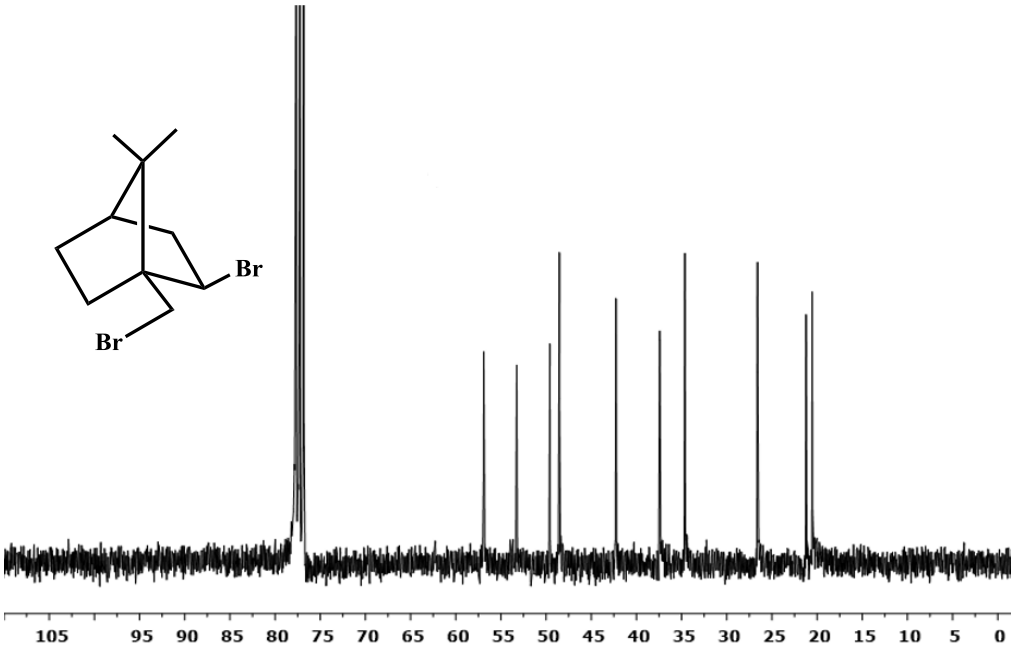


Figure 4.3. ^{13}C NMR of 2,10-dibromo-(-)-camphene.

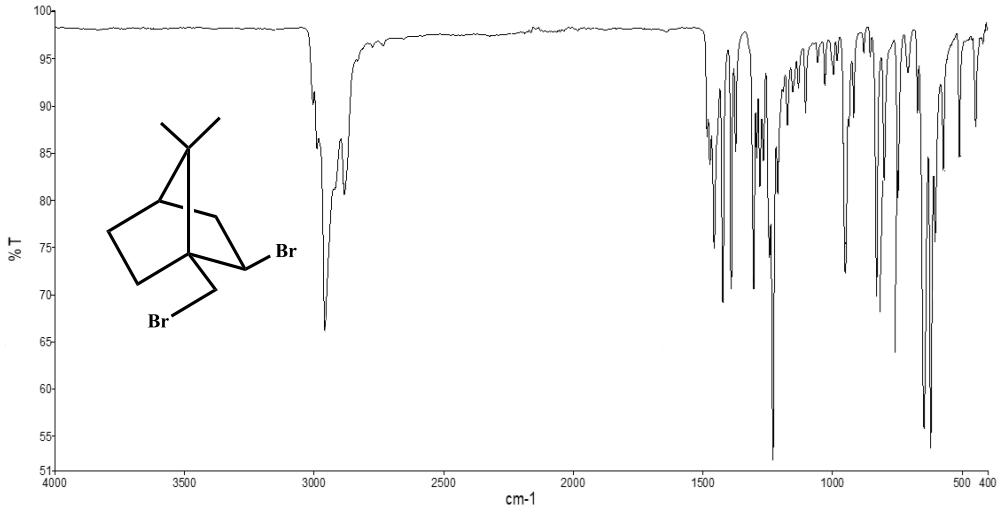


Figure 4.4. FTIR spectrum of 2,10-dibromo-(-)-camphene.

4.2. 2,10-Dibromo-(-)-Camphene Elimination Reaction with High Temperature

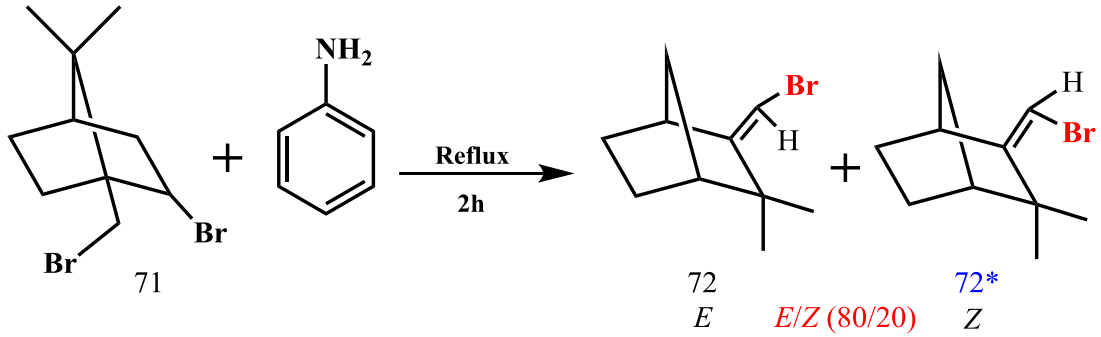


Figure 4.5. 2,10-Dibromo-(-)-camphene reactions with aniline.

In a single neck flask wrapped in thermal cotton fibers added (71) and aniline (10 gr, 30 mL) followed by stirring at the hotplate with reflux for 2 hours (Figure 4.5). The oily layer was extracted with methylene chloride (2 x 35 mL) and water (3 x 60 mL) after the organic phases' solution was extracted with hydrochloride acid 10%, the organic phases combined and dried with Na_2SO_4, and the solvent was removed to give black dense liquid-like crude product, applied to a silica gel column was done using hexane as the mobile phase solvent. An oily yellow liquid substance was obtained (4.2 g, 57.2% yield) for (*E*/*Z*) (1*S*,4*R*)-3-(bromomethylene)-2,2-dimethylbicyclo[2.2.1]heptane (72, 72*), it was determined consisted of (80/20), and all crudes were indicated by ^{1}H NMR analysis (Figure 4.6).

^{1}H NMR ($CDCl_3$, 300 MHz, ppm) δ_H 5.63 (s, 1H), 3.12 (dd, $J_{1ii,ex}$ = 3.61 Hz, $J_{1jj,en}$ = 3.61 Hz, 1H, H_1), 2.03 (dd, $J_{4,5ex}$ = 1.50 Hz, $J_{4,5en}$ = 1.5 Hz, 1H, H_4), 1.75-1.62 (m, 2H, H_{6ex}, and H_{6en}), 1.47-1.24 (m, 2H, H_{5ex}, and H_{5en}), 1.08 (s, 3H_{CH3}), 1.04 (s, 3H_{CH3}).

^{13}C NMR ($CDCl_3$, 75 MHz, ppm) δ_C 161.3, 94.2, 49.3, 45.3, 44.5, 37.0, 29.1, 27.2.

FTIR (cm^{-1}) ν = 3070 (C=C), 2957 (-CH_2), 2867 (C-H), 1458 (C-C), 1308 (C-Br).

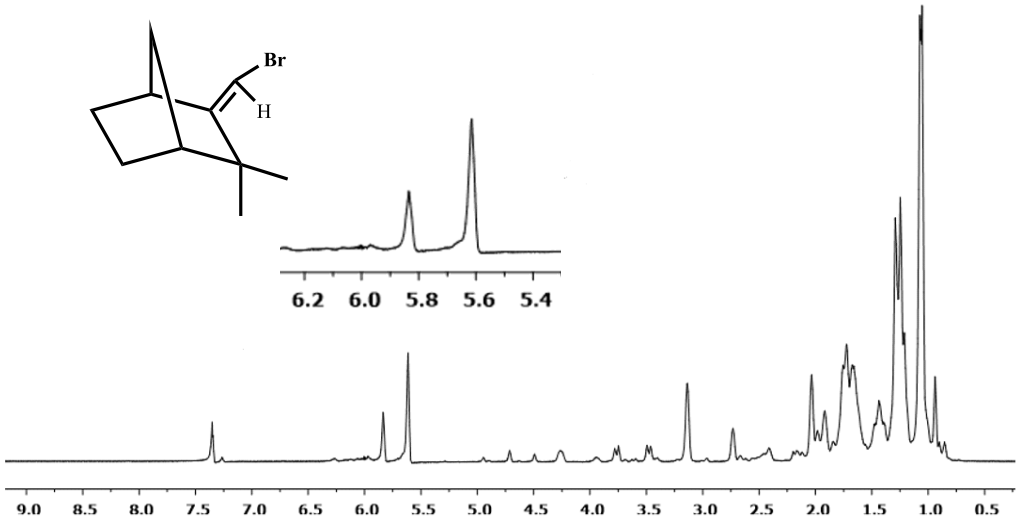


Figure 4.6. ^{1}H NMR of (*E*,*Z*)-3-(bromomethylene)-2,2-dimethylbicyclo [2.2.1] heptane.

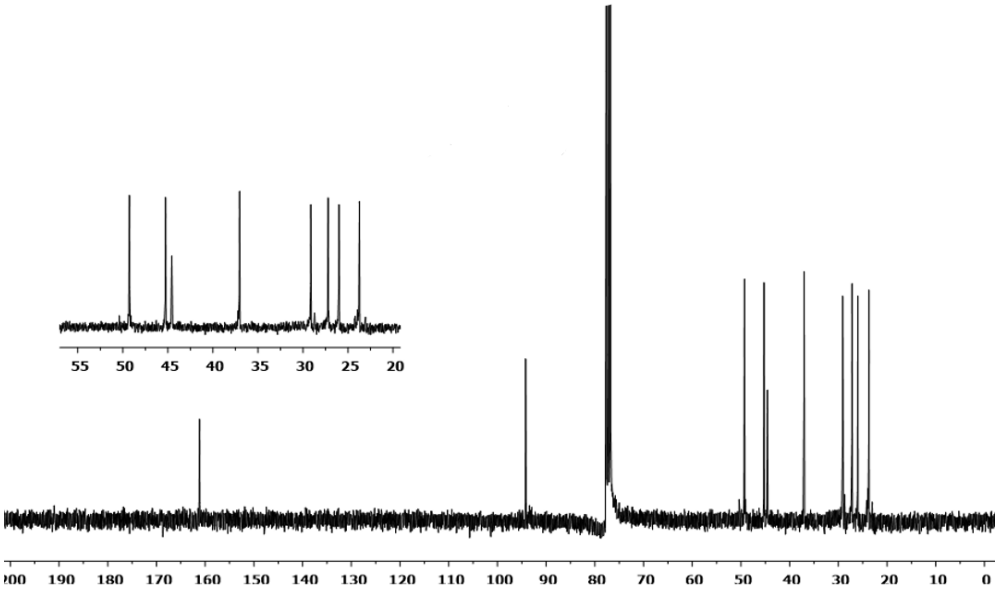


Figure 4.7. ^{13}C NMR of (*E*,*Z*)-3-(bromomethylene)-2,2-dimethylbicyclo[2.2.1] heptane.

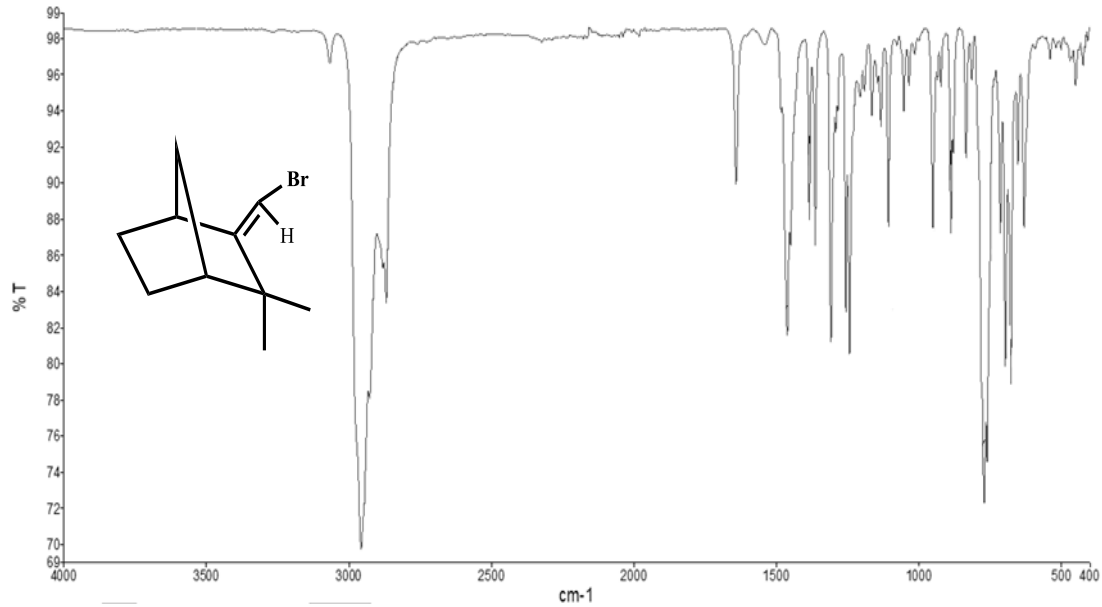


Figure 4.8. FTIR of (*E,Z*)-3-(bromomethylene)-2,2-dimethylbicyclo[2.2.1] heptane.

The mixture consisting of (72 and 72*) was reapplied with a silica gel column (15 mm diameter, 2000 mm length), and a new crude was collected as each 25 mL and looking at the ^{1}H NMR spectrum. The real crude of the *Z* isomer (72*) started coming from the 12th item and was isolated 20% as a colorless liquid. The ^{1}H NMR spectrum for *Z* isomer looking at in the olefinic region (5.8 ppm) a single singlet is observed it belongs to the H at the very end of the double bond. Other protons resonated in the aliphatic region (Figure 4.9, 4.10). In the ^{13}C NMR spectrum two out of ten signals are olefinic in the region of these bromines which resonates at 93 ppm and is attached to the carbon to which it is attached belongs. The leftmost signal is understood to belong to the quaternary olefinic carbon (Figure 4.11).

^{1}H NMR ($CDCl_3$, 300 MHz, ppm) δ_H 5.83 (s, 1H), 2.74 (dd, $J_{1ii,6ex}$ = 3.63 Hz, $J_{1jj,6en}$ = 3.63 Hz, 1H, H_1), 1.90 (dd, $J_{J4.5ex}$ = 2.71 Hz, $J_{4.5en}$ = 2.71 Hz, 1H, H_4), 1.91-1.86 (m, 2H, H_{6ex}, and H_{6en}), 1.76-1.58 (m, 2H, H_{5ex}, and H_{5en}), 1.05 (s, $3H_{CH3}$), 1.02 (s, $3H_{CH3}$).

^{13}C NMR ($CDCl_3$, 75 MHz, ppm) δ_C 158.7, 92.9, 50.4, 49.1, 43.7, 37.2, 28.8, 24.2, 23.9, 23.1.

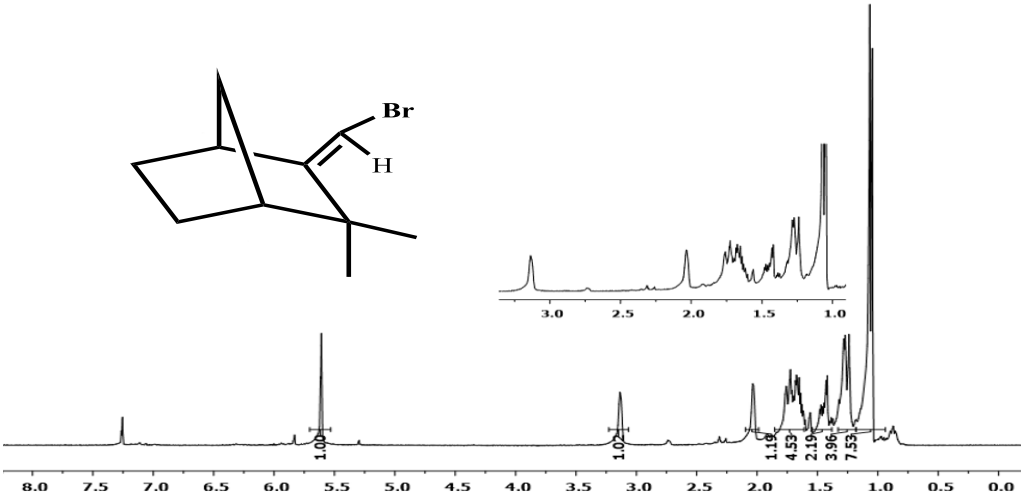


Figure 4.9. ^{1}H NMR of (*E*)-3-(bromomethylene)-2,2-dimethylbicyclo[2.2.1]heptane.

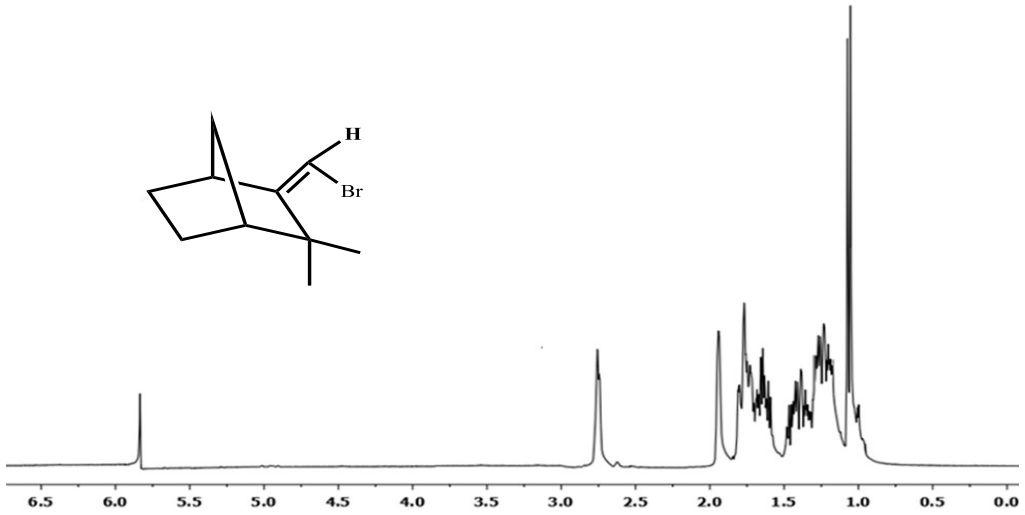


Figure 4.10. ^{1}H NMR of (*Z*)-3-(bromomethylene)-2,2-dimethylbicyclo[2.2.1]heptane.

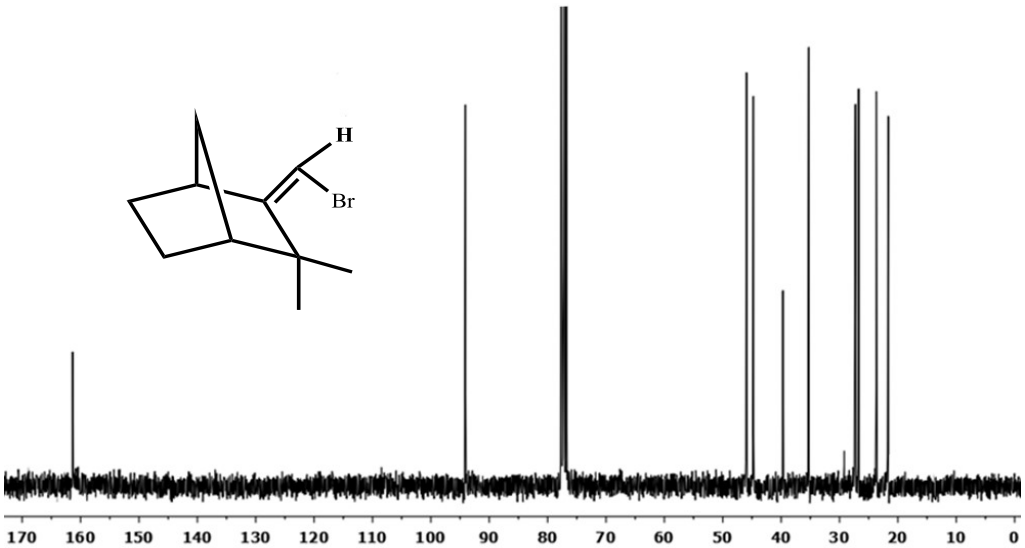


Figure 4.11. ^{13}C NMR of (*Z*)-3-(bromomethylene)-2,2-dimethylbicyclo[2.2.1] heptane.

It is obtained as the main product in the mixture formed by the elimination of normal addition products. It is the *E* isomer of the brominated derivatives of (-)-camphene. When the literature is examined, the main *Z* isomer is obtained as the product (Garamszegi and Schlosser, 1998; Sonawane et al., 1984). The brominated compound of camphene detailed descriptions of the related compounds in the literature, where the spectrum analyses are not given. The spectra of the *E* isomer are very similar to the *Z* isomer, *E* in the ^{1}H NMR spectrum olefinic H is seen in a slightly higher area (5.6 ppm) than *Z* this is in harmony with the structure. In the *Z* isomer, the H of the olefin is the same as the bridging methylene shares space (Figure 4.10). The thrust observed here is slightly lower than that of the olefinic hydrogen. Provides resonance in the field. Monobromo-(-)-camphene (72) has COSY, DEPT, and HETCOR analyses confirm the structure. Compound 72 in the olefinic and aliphatic region in the ^{13}C NMR spectrum a total of 10 signals are displayed two of these signals (161.2 and 94.2 ppm) are in the olefinic region (Figure 4.11).

4.3. (1*S*,4*R*)-3-Phenyl-2,2-Dimethyl [2.2.1] Heptane

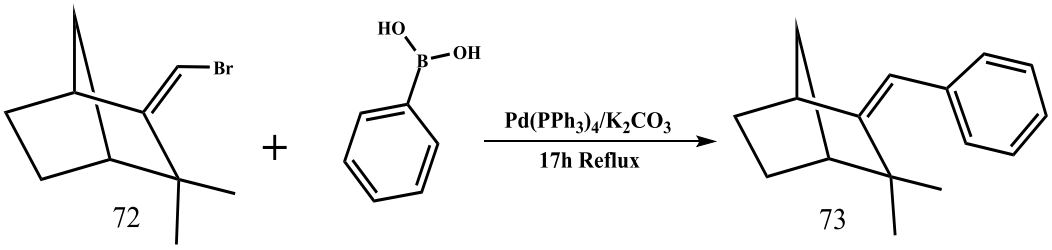


Figure 4.12. (1*S*,4*R*)-3-Phenyl-2,2-dimethyl[2.2.1]heptane reaction.

To an rt stirred added monobromo-(-)-camphene (72) phenylboronic acid (1eq, 1.2 eq) and (toluene/ethanol; 1:1) as a solvent, and the solution was stirred until the boronic acid wholly dissolved. To this solution, 0.5 eq was added $Pd(PPh_3)_4$ followed by an aqueous solution of potassium carbonate (0.95 mg in 8 mL H_2O). Mixed the ingregients for 3 min and then heated 110ºC to 17h reflux until N_2 gas showed complete consumption of the starting material (Figure 4.12). After allowing the reaction mixture to cool, the supernatant with chloroform was separated and removal of the solvent in vacuo, by silica gel chromatography (hexane gradient) gave 60% yield (0.3 g) yellow oil liquid (1*S*, 4*R*)-3-phenyl-2,2-dimethyl[2.2.1]heptane (73) was indicated by ^{1}H NMR analysis (Figure 4.13).

^{1}H NMR ($CDCl_3$, 300 MHz, ppm) δ_H 7.49-7.45 (m, $1H_{Hbenzene}$), 6.28 (m, $1H_{ethylene}$), 3.10(s, H, $H_{4cyclopentane}$), 1.98 (s, H, $H_{1cyclopentane}$), 1.69 (s, H, $H_{6cyclopentane}$), 1,50 (s, $H_{5cyclopentane}$), 1.40 (s, H, $H_{7cyclopentane}$), 1.08 (s, H_{CH3}).

^{13}C NMR ($CDCl_3$, 75 MHz, ppm) δ_C 195.5, 140.0, 129.6, 127.9, 126.5, 48.5, 44.0, 37.3, 35.5, 29.9.

FTIR (cm^{-1}) ν = 2955 (C-H_{Ph}), 2867 (-CH_2), 1560 (C=C_{Ph}), 1459 (C=C), 749 (-CH_3).

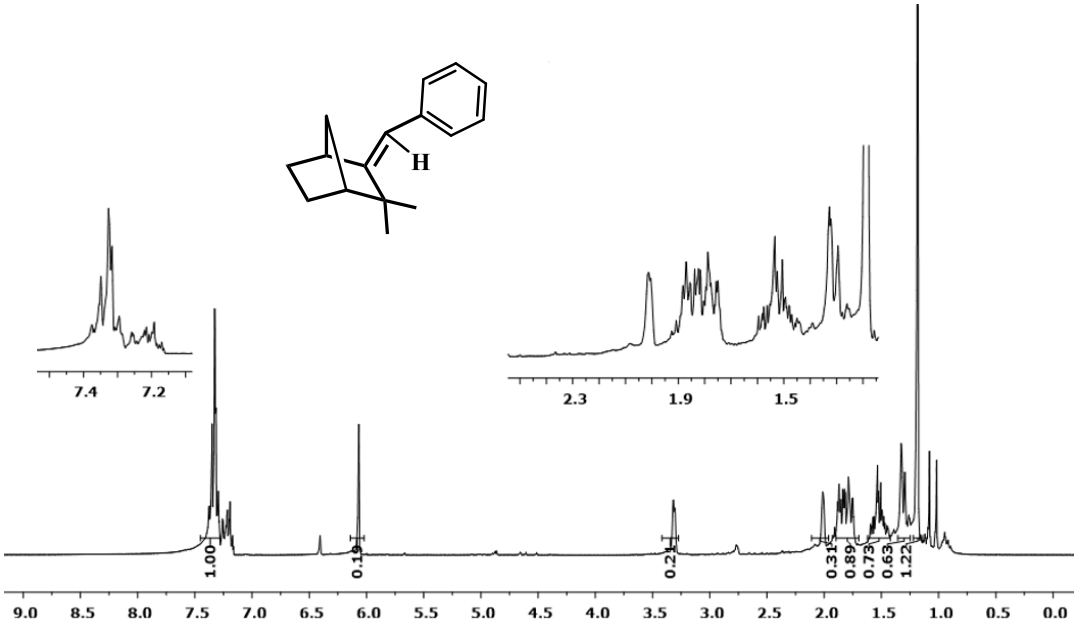


Figure 4.13. ^{1}H NMR of (1*S*,4*R*)-3-phenyl-2,2-dimethyl[2.2.1]heptane.

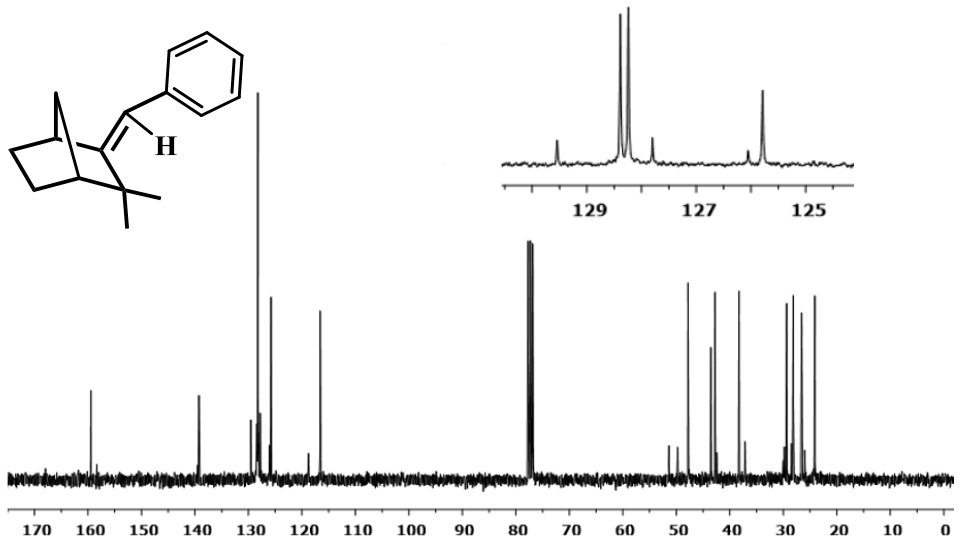


Figure 4.14. ^{13}C NMR of (1*S*,4*R*)-3-phenyl-2,2-dimethyl[2.2.1]heptane.

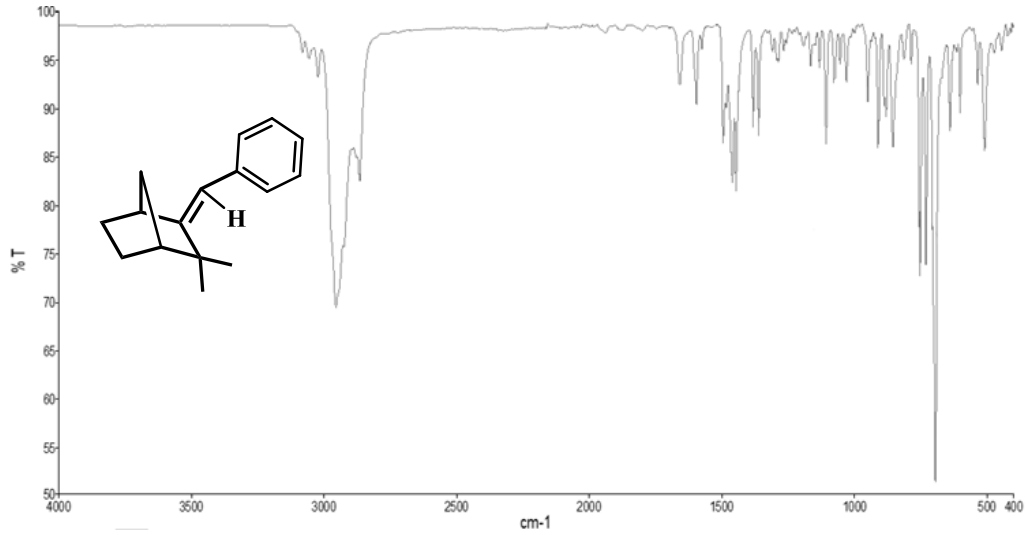


Figure 4.15. FTIR of (1*S*,4*R*)-3-phenyl-2,2-dimethyl[2.2.1]heptane.

4.4. (1*S*,4*R*)-3-(4-Thiomethylphenyl)-2,2-Dimethyl[2.2.1]Heptane

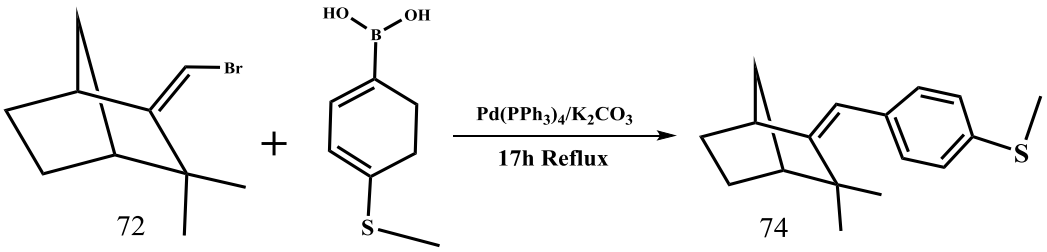


Figure 4.16. (1*S*,4*R*)-3-(4-Thiomethylphenyl)-2,2-dimethyl[2.2.1]heptane reaction.

To an rt stirred 1 eq compound 72 was added to 4-thiomethylphenylboronic acid (1.2 eq) with the (toluene/ethanol; 1:1) as a solvent, and the solution was stirred until the boronic acid wholly dissolved. To this solution, 0.5 eq was added $Pd(PPh_3)_4$ followed by an aqueous solution of potassium carbonate (0.95 mg, in 8 mL H_2O). Mixed the ingregients for 3 min and then heated 110ºC to 17h reflux until N_2 gas showed complete consumption of the starting material (Figure 4.16). After allowing the reaction mixture to cool, the supernatant with chloroform was separated and the removal of the solvent in vacuo, by silica gel chromatography (hexane gradient) gave the title compounds a 97% yield (0.4 g) yellow crystal (1*S*,4*R*)-3-(4-thiomethylphenyl)-2,2-dimethyl[2.2.1]heptane (74) was indicated by ^{1}H NMR analysis (Figure 4.17).

^{1}H NMR ($CDCl_3$, 300 MHz, ppm) δ_H 7.35-7.31 (m, $H_{benzene}$), 6.28 (m, $H_{ethylene}$), 3.21 (s, $H_{4cyclopentane}$), 2.50 (m, H_{SCH3}), 1.95 (s, $H_{6cyclopentane}$), 1.49 (s, $H_{5cyclopentane}$), 2.01 (s, $H_{1cyclopentane}$), 1.08 (m, H_{CH3}).

^{13}C NMR ($CDCl_3$, 75 MHz, ppm) δ_C 159.5, 140.0, 127.9, 127.8, 125.5, 116.3, 78.5, 47.0, 45.3, 38.5, 29.9, 19.8.

FTIR (cm^{-1}) ν = 2940 (C-H_{Ph}), 2865 (C-H_{methyl}) 1655 (C=C), 1490 (C=C_{Ph}), 1099 (C-S), 862 (C-H).

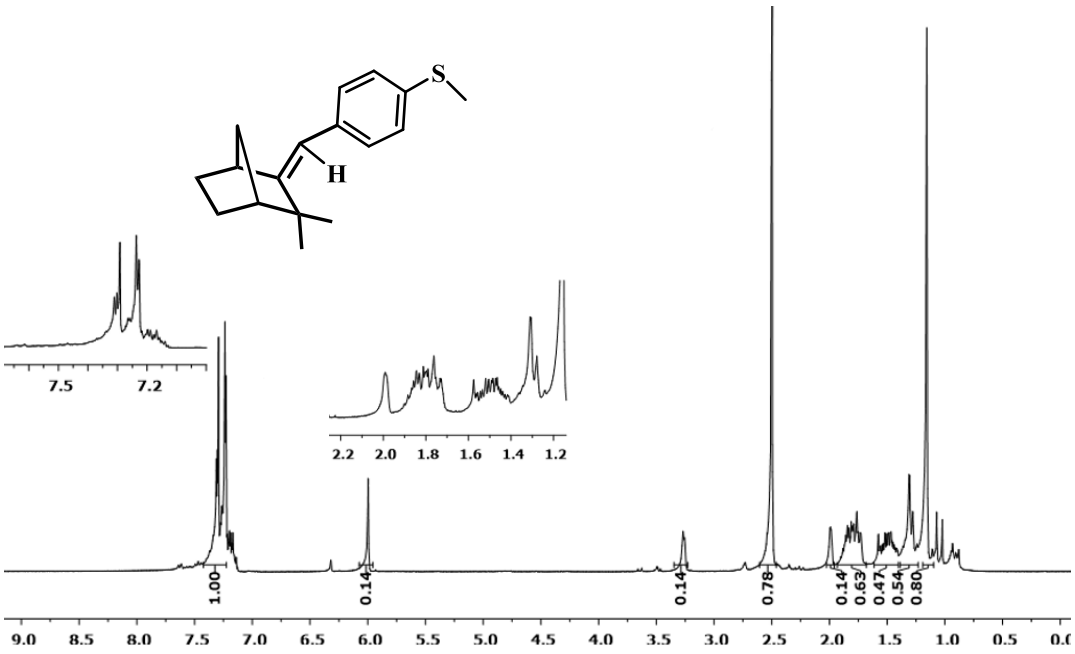


Figure 4.17. ^1H NMR of (1*S*,4*R*)-3-(4-thiomethylphenyl)-2,2-dimethyl[2.2.1]heptane.

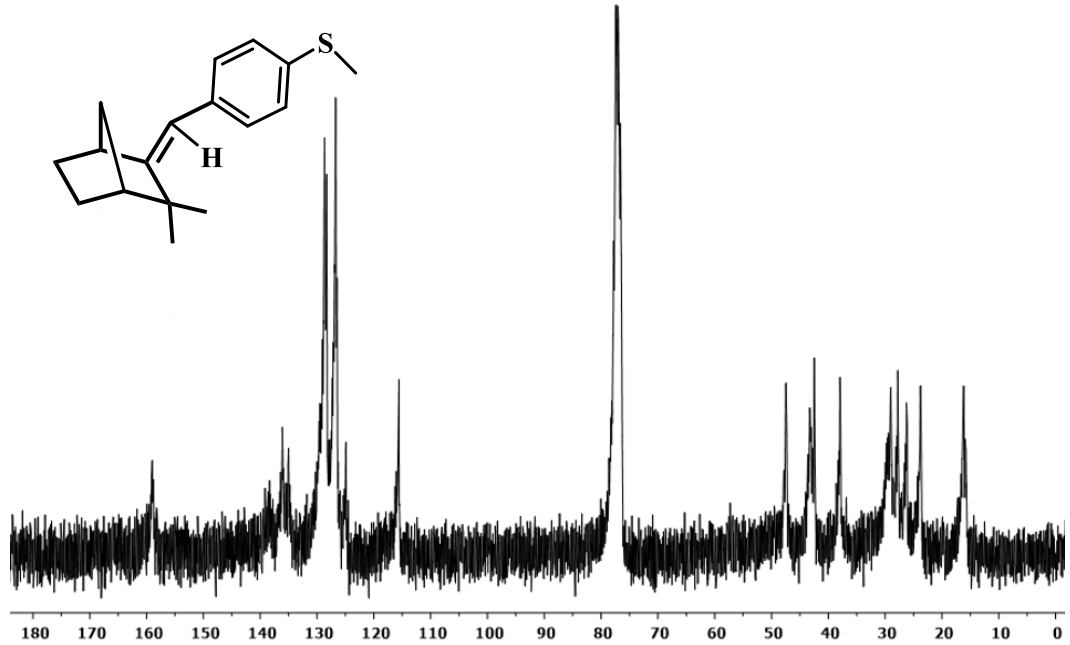


Figure 4.18. ^{13}C NMR of (1*S*,4*R*)-3-(4-thiomethylphenyl)-2,2-dimethyl[2.2.1] heptane.

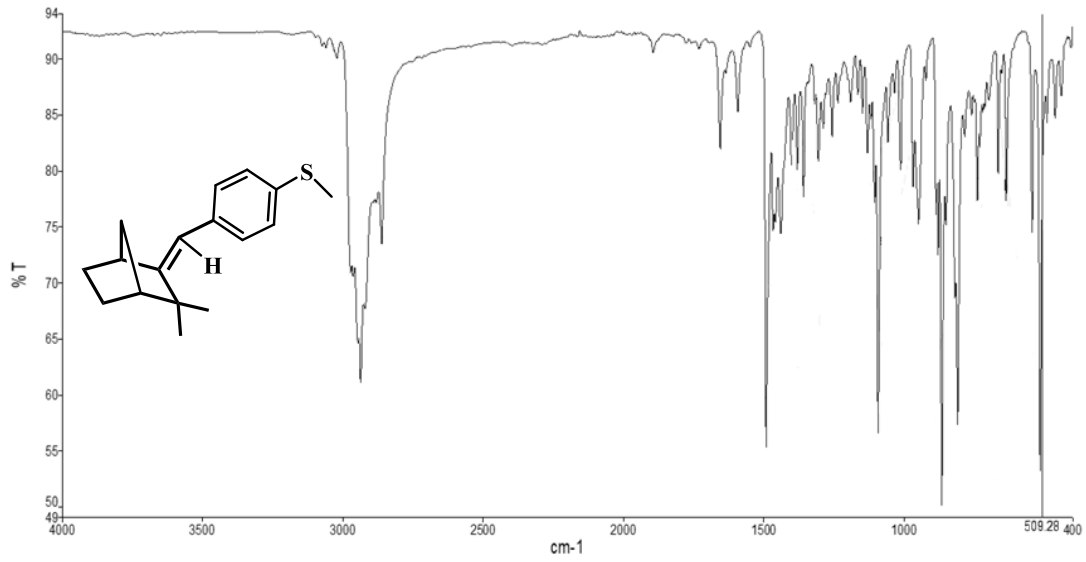


Figure 4.19. FTIR of (1*S*,4*R*)-3-(4-thiomethylphenyl)-2,2-dimethyl[2.2.1]heptane.

4.5. (1*S*,4*R*)-3-(4-Ethylphenyl)-2,2-Dimethyl[2.2.1]Heptane

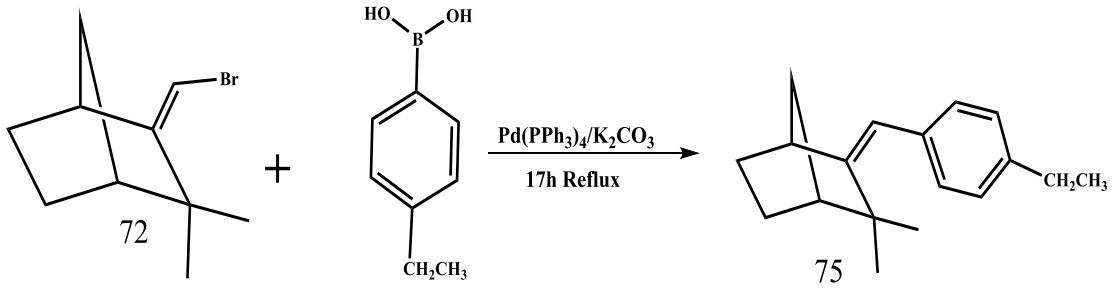


Figure 4.20. (1*S*,4*R*)-3-(4-Ethylphenyl)-2,2-dimethyl[2.2.1]heptane.

To an rt stirred compound 72 (1 eq.) was added to 4-ethylphenylboronic acid (1.2 eq.) with the (toluene/ethanol; 1:1) as a solvent, and the solution was stirred until the boronic acid wholly dissolved. To this solution, 0.5 eq. was added $Pd(PPh_3)_4$ followed by an aqueous solution of potassium carbonate (0.95 mg, in 8 mL H_2O). Mixed the ingredients for 3 min and then heated 110ºC to 17h reflux until N_2 gas showed complete consumption of the starting material (Figure 4.20). After allowing the reaction mixture to cool, the supernatant with chloroform was separated and removal of the solvent was in vacuo, by silica gel chromatography (hexane gradient) to give 96% yield (0.58 g) yellow oil liquid (1*S*,4*R*)-3-(4-ethylphenyl)-2,2-dimethyl[2.2.1]heptane was indicated by ^1H NMR analysis (Figure 4.21).

^{1}H NMR ($CDCl_3$, 300 MHz, ppm) δ_H 7.23 ($H_{12benzene}$), 7.21 (m, $H_{13benzene}$), 6.16 (m, $H_{ethylene}$), 3.45 (m, H_{CH2}), 2.64 (m, $H_{H4cyclopentane}$), 1.98 (s, $H_{H1cyclopentane}$), 1.67 (m, $H_{H6cyclopentane}$), 1.54 (s, $H_{H5cyclopentane}$), 1.36 (m, $2H_{CH3}$) and 1.38 (m, H_{CH3}).

^{13}C NMR ($CDCl_3$, 75 MHz, ppm) δ_C 159.1, 143.5, 136.2, 128.5, 116.5, 49.5, 43.3, 39.5, 29.2, 26.8.

FTIR (cm^{-1}) ν = 2965 ($-CH_3$), 2896 ($-CH_3$), 2832 ($-CH_2$), 1608 (C=C), 1508 (C=C), 1239 (C-C).

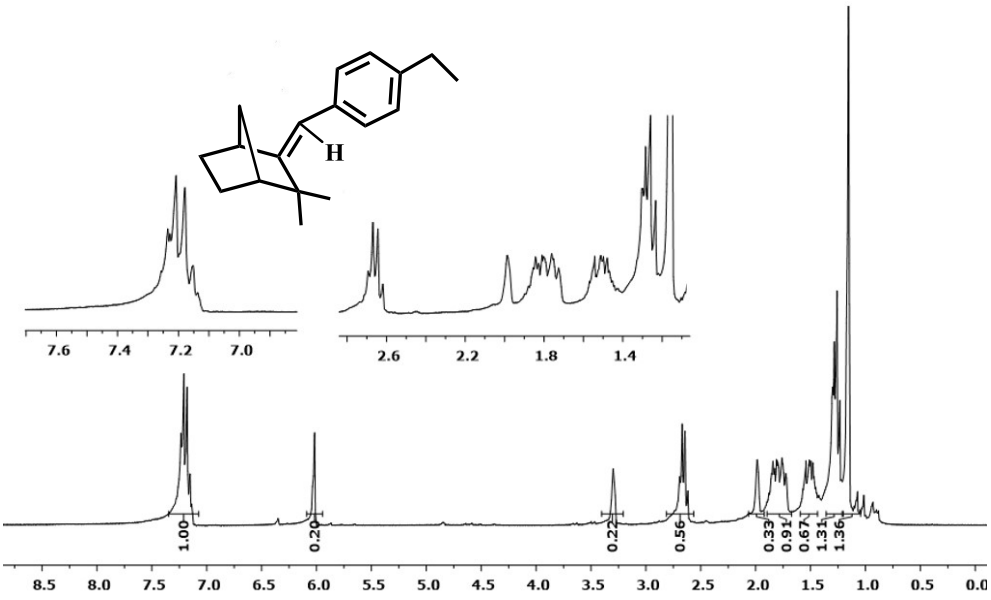


Figure 4.21. ^{1}H NMR of (1*S*,4*R*)-3-(4-ethylphenyl)-2,2-dimethyl[2.2.1]heptane.

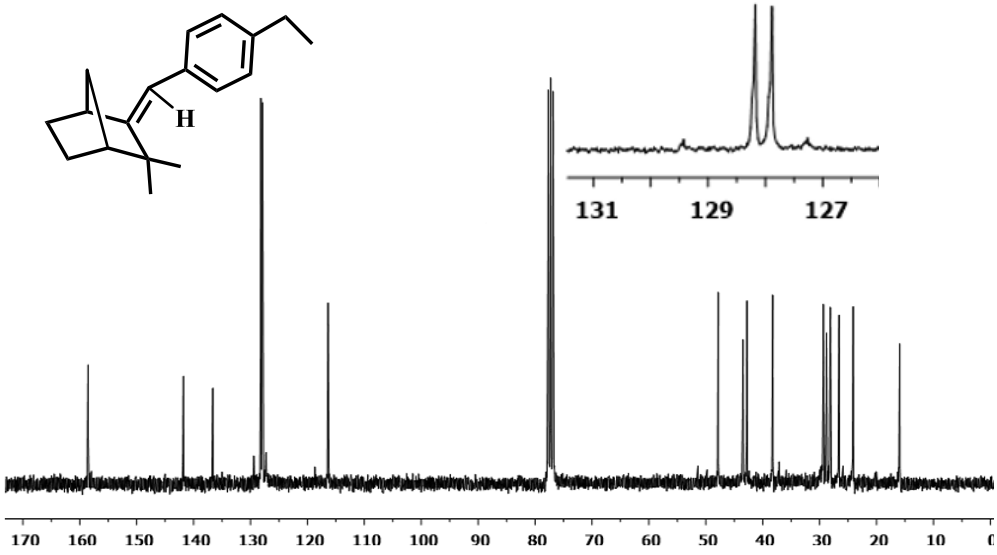


Figure 4.22. ^{13}C NMR of (1*S*,4*R*)-3-(4-ethylphenyl)-2,2-dimethyl[2.2.1]heptane.

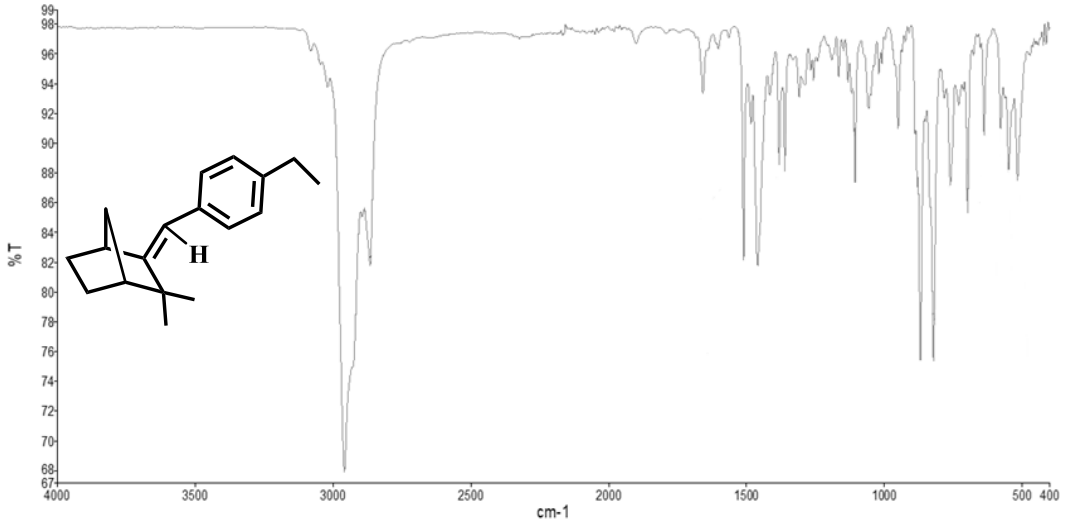


Figure 4.23. FTIR of (1*S*,4*R*)-3-(4-ethylphenyl)-2,2-dimethyl[2.2.1]heptane.

4.6. (1*S*,4*R*)-3-(4-Methoxyphenyl)-2,2-Dimethyl[2.2.1]Heptane

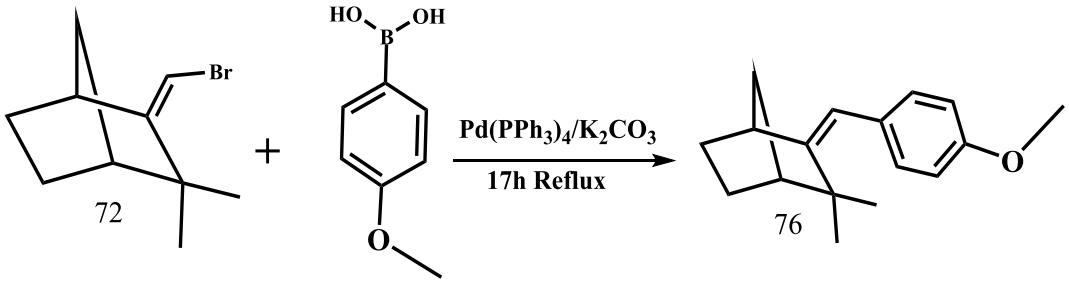


Figure 4.24. (1*S*,4*R*)-3-(4-Methoxyphenyl)-2,2-dimethyl[2.2.1]heptane reaction.

To an rt stirred compound 72 (1 eq.) was added to 4-methoxyphenylboronic acid (1.2 eq.) with the (toluene/ethanol; 1:1) as a solvent and the solution was stirred until the boronic acid wholly dissolved. To this solution, 0.5 eq. was added $Pd(PPh_3)_4$ followed by an aqueous solution of potassium carbonate (0.75 mg, in 8 mL H_2O). Mixed the ingredients for 3 min and then heated 110ºC to 17h reflux until N_2 gas showed complete consumption of the starting material (Figure 4.24). After allowing the reaction mixture to cool, the supernatant with chloroform was separated and removal of the solvent in vacuo, by silica gel chromatography (hexane gradient) to give 85% yield (0.35 g) of white crystal-like cotton. (1*S*,4*R*)-3-(4-Methoxyphenyl)-2,2-dimethyl[2.2.1]heptane (76) was indicated by ^{1}H NMR analysis (Figure 4.25).

^{1}H NMR ($CDCl_3$, 300 MHz, ppm) δ_H 7.20 (m, $H_{H12benzene}$), 7.01 (m, $H_{H13benzene}$), 5.99 (s, $H_{ethylene}$), 3.82 (m, H_{OCH3}), 3.19 (m, $H_{H4cyclopentane}$), 1.90 (s, $H_{H1cyclopentane}$), 1.70 (s,

$H_{H6cyclopentane}$), 1.55 (s, $H_{H5cyclopentane}$), 1.42 (s, $H_{H7cyclopentane}$), 1.25 (s, $H_{H5cyclopentane}$), 1.15 (m, H_{CH3}).

^{13}C NMR ($CDCl_3$, 75 MHz, ppm) δ_C 158.9, 131.0, 130.9, 116.5, 113.5, 55.8, 48.8, 43.5, 39.1, 29.1, 26.9, 23.8.

FTIR (cm^{-1}) ν = 2949 ($-CH_3$), 2865 ($-CH_2$), 2831 (-CH), 1608 ($C=C_{Ph}$), 1509 (C=C), 1240 (C-O).

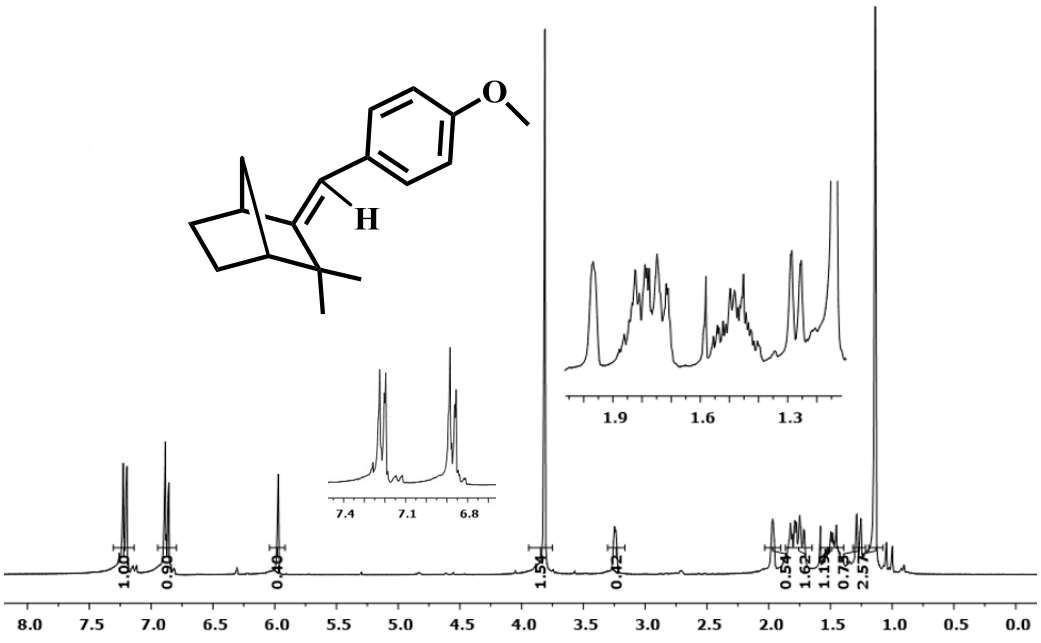


Figure 4.25. ^1H NMR of (1*S*,4*R*)-3-(4-methoxyphenyl)-2,2-dimethyl[2.2.1]heptane.

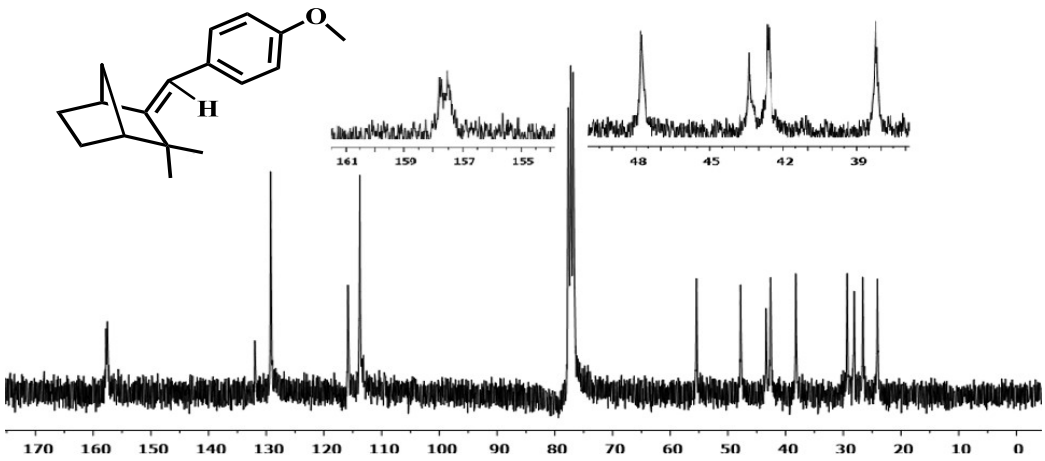


Figure 4.26. ^{13}C NMR of (1*S*,4*R*)-3-(4-methoxyphenyl)-2,2-dimethyl[2.2.1]heptane.

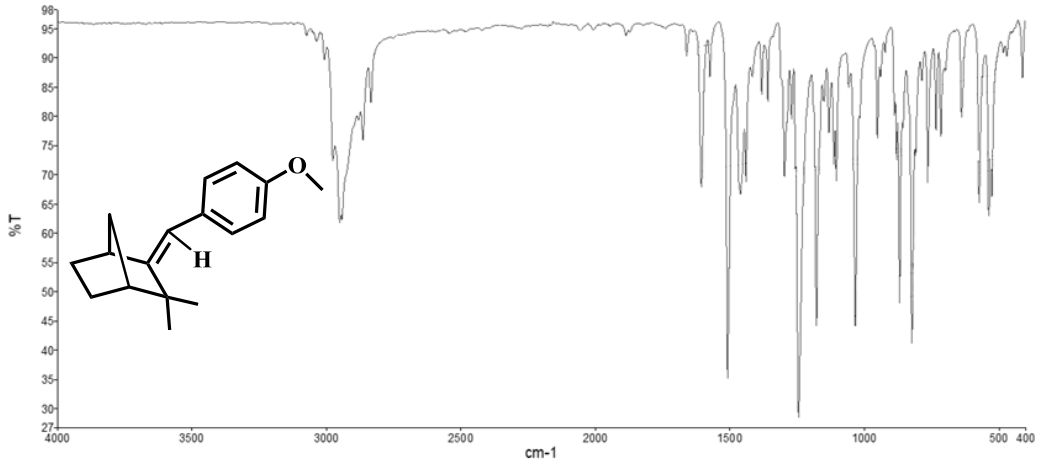


Figure 4.27. FTIR of (1*S*,4*R*)-3-(4-methoxyphenyl)-2,2-dimethyl[2.2.1]heptane.

4.7. (1*S*,4*R*)-3-(4-Trifluoromethoxylphenyl)-2,2-Dimethyl[2.2.1]Heptane

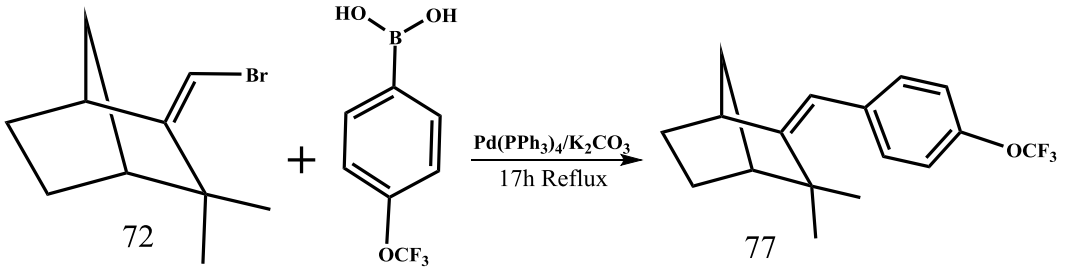


Figure 4.28. (1*S*,4*R*)-3-(4-Trifluoromethoxylphenyl)-2,2-dimethyl[2.2.1] heptane reaction.

To an rt stirred compound 72 (1 eq.) was added to 4-trifluoromethoxyphenylboronic acid (1.2 eq.) with the (toluene/ethanol; 1:1) as a solvent and the solution was stirred until the boronic acid wholly dissolved. To this solution, 0.5 eq. was added $Pd(PPh_3)_4$ followed by an aqueous solution of potassium carbonate (0.75 mg, in 8 mL H_2O). Mixed the ingregients for 3 min and then heated 110ºC to 17h reflux until N_2 gas showed complete consumption of the starting material (Figure 4.28). After allowing the reaction mixture to cool, the supernatant with chloroform was separated and removal of the solvent in vacuo, by silica gel chromatography (hexane gradient) to give 96% yield (0.41 g) yellow oil liquid (1*S*,4*R*)-3-(4-methoxyphenyl)-2,2-dimethyl[2.2.1]heptane (77) was indicated by ^{1}H NMR analysis (Figure 4.29).

^{1}H NMR (CDCl3, 300 MHz, ppm) δ_H 7.53 (m, $H_{12benzene}$), 7.19 (m, $H_{13benzene}$), 6.02 (m, $H_{ethylene}$), 3.22 (s, $H_{H4cyclopentane}$), 1.95 (s, $H_{H1cyclopentane}$), 1.83(s, $H_{H6cyclopentane}$), 1.81 (s, $H_{H5cyclopentane}$), 1.56 (s, $H_{H7cyclopentane}$), 1.35 (s, $H_{H7cyclopentane}$), 1.09 (m, H_{CH3}).

^{13}C NMR ($CDCl_3$, 75 MHz, ppm) δ_C 169.0, 157.5, 138.0, 129.5, 120.5, 48,5, 43.3, 37.5, 35.5, 29.2, 26.2

FTIR (cm^{-1}) ν = 2960 ($-CH_3$), 2865 ($-CH_2$), 1503 ($C=C_{Ph}$), 1462 (C=C), 1258 (C-O), 873 (C-F).

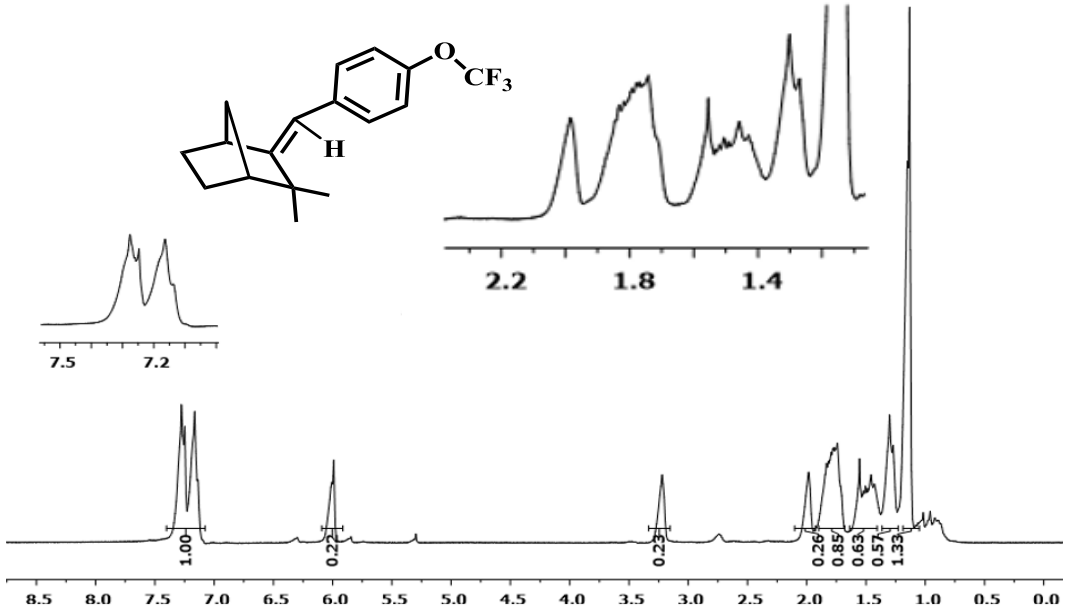


Figure 4.29. ^{1}H NMR of (1*S*,4*R*)-3-(4-trifluoromethoxylphenyl)-2,2-dimethyl[2.2.1] heptane.

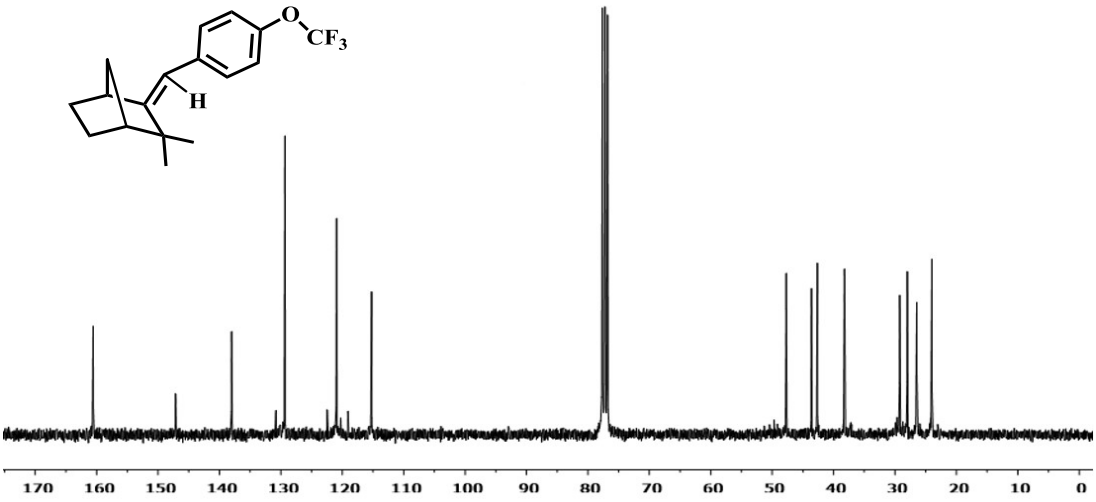


Figure 4.30. ^{13}C NMR of (1*S*,4*R*)-3-(4-trifluoromethoxylphenyl)-2,2-dimethyl[2.2.1] heptane.

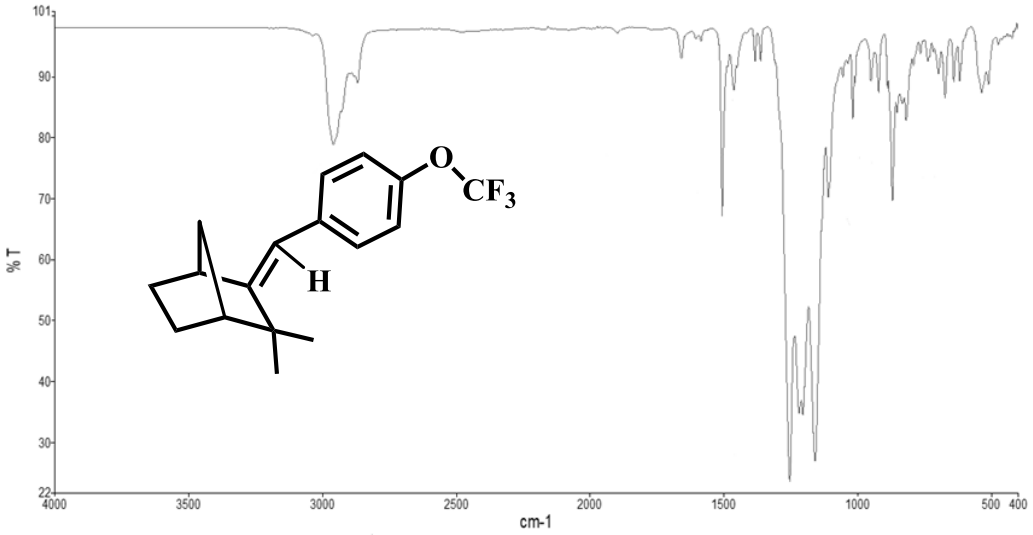


Figure 4.31. FTIR of (1*S*,4*R*)-3-(4-trifluoromethoxylphenyl)-2,2-dimethyl[2.2.1] heptane.

4.8. Optical Rotation

All synthesized compounds are prepared 1 g/10 mL in dichloromethane you apply the equation to find values of the obtained flip angle measured at 23°C using sodium D-line light (wavelength of 589 nm), 0.5 dm length of the polarimeter cell (Table 4.1).

Table 4.1. Optical rotation of (-)-camphene derivatives.

Compound	71	72	73	74	75	76	77
α	0.15	-0.26	-0.59	-0.09	-0.73	-0.15	-0.10
$[\alpha]_{\lambda}^{T}$	30	-52	-118	-90	-146	-30	-100

4.9. Antibacterial Assays

4.9.1. Evaluation according to NCI-60 screening methodology for a new (-)-camphene derivatives

To determine the cytotoxicity effect of compounds 71-77 used the MTT assay on lung and bone cancer together with normal lung and bone cells, in the NCI-60 screening methodology, high LC_{50} values demonstrating that 71-77 have less cytotoxic effects are desirable. At the same time, low GI_{50} and TGI values which are also preferable features in the screening methodology indicate that the test substances have a greater cytostatic effect. Accordingly, the findings demonstrated that 47 and 72 (GI_{50} value 1.07-1.68, TGI value 2.36- 55.97, and LC_{50} value > 500 µg/mL for A549, Calu1, and H1650 lung cancer cells) were capable to induce an anticancer effect without affecting normal cells (GI_{50} value 1.16-1.27, TGI value 2.66-23.72, and LC_{50} value > 500 µg/mL

for Beas2B normal lung cells) (Table 4.2). On the other hand, when considering all treated bone cancer cell lines, the 76 and 72 molecules with strong anticancer effect (GI_{50} value 1.02-1.63, TGI value 1.22-33.47, and LC_{50} value 6.10 - > 500 μg/mL for MG63, Saos2, and SW1353 bone cancer cells) is the most convenient one for further preclinical studies because they exhibit low cytotoxicity towards normal chondrocyte HC cells (GI_{50} value 1.06-2.14, TGI value 2.26-39.79, and LC_{50} value > 500 μg/mL) (Table 4.3). The anticancer effect exhibited by these molecules (in the range of TGI values 2.36-21.52 μg/mL) was better than the control drug, 5FU, in H1650 cancer cells (Table 4.2). When considering Calu1 cancer cells, these molecules (in the range of TGI values 4.30-71.79 μg/mL) exhibited similar or better anticancer effects than the control drug, 5FU (Table 4.2). The compound 71, different from the others, was found to be more effective on cancer cells but also it was very toxic against Beas 2B normal lung cells (LC_{50} value 101.9 μg/mL), which precluded the use of it (Table 4.2). When evaluating 71, 72, and 76 molecules together with MG63 bone cancer cells, it was expressed that these molecules exhibited strong antiproliferative effects (in the range of TGI values 1.46-30.65 μg/mL) (Table 4.3). When considering Saos2 cancer cells, these molecules (in the range of TGI values 1.22-103.5 μg/mL) exhibited similar or better anticancer effects than the control drug, 5FU (Table 4.3). When examining Table 4.3, it was seen that the anticancer activity exhibited by subjected molecules except for 73 and 77 was found at a similar or higher level than the control anticancer drug 5FU in SW1353 cancer cells (TGI values 1.42-20.25 μg/mL). When compared to positive control 5FU, it was seen that 74 was very toxic against HC normal chondrocyte cells (LC_{50} value 313.73 μg/mL) despite it being found to be more affected on bone cancer cells (Table 4.2). This situation limits the use of the 74 agents. If these 71 and 74 molecules' toxic effects against lung and chondrocyte normal cells, respectively, are reduced by chemical by modifying, they may be a good option for cancer treatment.

Table 4.2. GI_{50}, TGI, and LC_{50} values for tested compounds against A549, Calu1, H1650, and Beas2B*

Compounds (*μg/mL*)	A549**			Calu1**			H1650**			Beas2B**		
	GI50	**TGI**	**LC50**	**GI50**	**TGI**	**LC50**	**GI50**	**TGI**	**LC50**	**GI50**	**TGI**	**LC50**
74	1.13	3.59	>500	1.27	15.5±0.8	>500	1.07	2.36	>500	1.16	2.66	>500
76	1.30	6.00	>500	5.76	>500	>500	1.12	2.77	>500	1.05	2.01	>500
75	1.32	>500	>500	1.02	13.77±0.7	>500	1.78	21.52±1.0	>500	1.06	2.07	>500
71	1.68	5.08	160.6±5.3	1.15	9.41	>500	1.51	7.50	>500	1.04	1.56	101.9±5.0
77	3.51	>500	>500	1.07	71.79±2.7	>500	1.28	4.58	>500	1.61	6.91	>500
73	3.67	>500	>500	1.00	12.85±0.6	>500	1.37	5.71	>500	1.61	10.86±0.5	>500
72	1.68	55.97±2.0	>500	1.21	4.30	>500	1.39	4.42	>500	2.27	23.72±1.1	>500
5FU	1.51	48.3±1.5	478.0±17.2	1.52	62.1±3.0	452.4±17.1	1.53	52.1±2.7	420.9±16.1	1.40	45.2±2.8	427.5±15.0

*Percent inhibition noted is mean values ± SDs of three independent measures.
** If percent inhibition is smaller than 10, the SD value is <0.5.

Table 4.3. GI_{50}, TGI, and LC_{50} values for tested compounds against MG63, Saos2, SW1353, and HC*

Compounds (*μg/mL*)	MG63**			Saos2**			SW1353**			HC**		
	GI50	**TGI**	**LC50**	**GI50**	**TGI**	**LC50**	**GI50**	**TGI**	**LC50**	**GI50**	**TGI**	**LC50**
74	1.14	2.30	184.1±7.1	1.1	1.84	35.75±1.4	1.03	1.39	19.23±0.9	1.12	2.28	313.73±14.0
76	1.03	1.46	43.32±1.8	1.02	1.22	6.10	1.04	1.42	17.92±0.9	1.06	2.26	>500
75	1.52	>500	>500	2.33	103.5±4.2	>500	1.22	9.98	>500	6.47	>500	>500
71	1.37	30.65±1.5	>500	1.36	3.39	124.0±4.9	1.46	20.25±1.0	>500	2.38	>500	>500
77	3.69	>500	>500	1.77	35.15±1.8	>500	1.66	>500	>500	7.95	>500	>500
73	1.67	>500	>500	1.68	38.33±1.7	>500	1.18	>500	>500	4.56	>500	>500
72	1.35	8.90	>500	1.63	33.47±1.6	>500	1.53	6.74	>500	2.14	39.79±1.3	>500
5FU	1.53	40.7±2.8	451.2±19	1.62	53.1±2.5	423.4±18	1.52	44.5±1.8	417.0±14	1.65	56.5±2.0	423.4±17

*Percent inhibition noted is mean values ± SDs of three independent measures.
** If percent inhibition is smaller than 10, the SD value is <0.5.

When the findings from both normal and cancer cells were reviewed in detail, it was vigorously speculated that compound 72 is more cancer-specific for bone and lung cancer types, and makes it a most convenient one for further preclinical studies.

4.9.2. Cytotoxic activity of the new (-)-camphene derivatives

A new (-)-camphene derivatives were measured with the help of an LDH cytotoxicity kit, the leaks enzyme from the cell membrane destroyed by the tested agent and is rapidly released into the cell media supernatant, a crucial indicator of cells going through necrosis, apoptosis, and other types of cell death. With the help of an LDH cytotoxicity kit we measured the cytoplasmic LDH activity. In this study, cytotoxicity induced by (-)-camphene compounds 71-77 were measured TGI concentrations. So compound 72 at TGI concentration that were found to function well in the test MTT proliferation and similar cytotoxicity a varying values of 17-19% for cancer and normal cell lines compared to 5FU drug control. In addition, the other effective molecules, 74 for Beas2B lung normal cells (18.0) and 76 for HC chondrocyte normal cells (19.0), exhibited acceptable toxicity (Table 4.4). The tested cytotoxic effects of (-)-camphene derivatives, were found to be effective in MTT proliferation assay, on lung cancer cells (A549, Calu1, and H1650) and bone cancer cells (Saos2, MG63, and SW1353) were 2.7-25.5% and 2.4-29.8% at TGI concentration, respectively (Table 4.4).

Table 4.4. % Cytotoxicity for tested compounds at TGI concentrations against the cells*

Compounds	A549	Calu1	H1650	MG63	Saos2	SW1353	Beas2B	HC
74	17.1	18.2	19.6	25.1	29.8	27.1	18.0	20.3
76	22.0	3.6	25.5	17.8	18.6	19.0	22.1	19.0
75	3.8	19.1	19.1	2.4	8.0	23.3	21.2	2.7
71	23.4	23.5	22.0	17.0	26.1	20.4	29.8	3.5
77	2.7	14.2	23.0	2.4	18.0	3.1	22.4	4.0
73	3.0	18.2	20.2	3.8	17.2	3.0	23.0	2.3
72	17.5	18.4	19.3	18.0	19.1	17.7	19.5	17.2
5FU	18.5	16.3	15.5	17.1	14.2	13.1	16.5	15.0

*Percent cytotoxicity was noted as mean values ± SDs of three independent measures (< 2.0).

The MTT and LDH assay results, which show the therapeutic and cytotoxic features, respectively, were evaluated together, it was vigorously expressed that compounds 72, 74, and 76 turns out the most optimal effect of cytotoxic against, antiproliferative

cancer and normal cells compared to the positive control, 5FU (Table 4.2, 4.3, and 4.4).

4.9.3. DNA degradation

In the apoptotic process, a specific caspase-activated DNase leads to a particular cleavage of DNA at internucleosomal linker sites resulting in fragments (~200) of base pairs like ladders. Therefore, apoptotic DNA appears like a ladder shape on an agarose gel. To detect DNA fragments used the agarose gel DNA laddering method helps to examine apoptotic status in a cell, the result of DNA laddering test of compounds 71-77 induced the formation of DNA fragments in MG63, A549, and Beas2B cells compared to the untreated cells (Figure 4.32). However, it should be said that there is less fragmentation in the control cells (Beas2B) compared to cancer cells (MG63 and A549). This effect highlights that (-)-camphene molecules have more anticancer effects on cancer cells than normal cells and in untreated cells (well 1), there were no DNA fragmentation, and intact genomic DNA was placed near the well. Overall, the 71-77 may trigger an apoptotic cascade in the cell and cause the appearance of apoptotic DNA fragmentation.

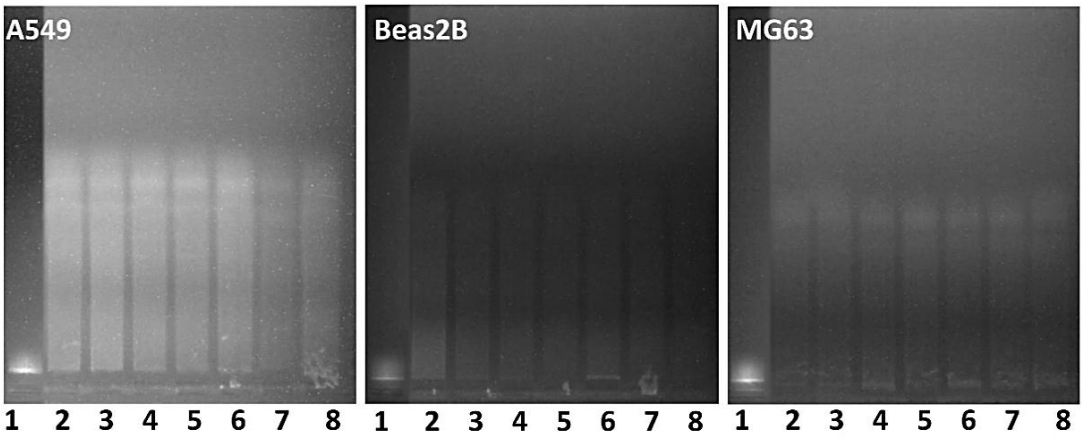


Figure 4.32. Effects of the 74-(2), 76-(3), 75-(4), 71-(5), 77-(6), 73-(7) and 72-(8) on DNA laddering in A549, Beas2B and MG63 cell lines. Well (1) is an untreated control for all cells.

4.9.4. Effect of substances on cell migration

A new anticancer agents that make the cell migration rate is a significant determinant of cancer cells, the migration capacity of the cancer cells helps them escape from the apoptosis mechanism. Along with invasion and angiogenesis, migration, is the most crucial stage of metastasis. Therefore, one of the aims of newly developed (-)-camphene molecules is to significantly reduce the migration capacity of cancer cells.

Migration test results of compounds 71, 75, 76, and 77 that were found to be effective during the study are as follows. According to the time-dependent migration test 71-77 used at TGI concentration significantly suppressed the migration capacity of cancer cells (A549 and MG63) compared to DMSO group under control (Figures 4.33 and 4.34).

Table 4.5. Migration analysis of the molecules with the ImageR

% Area	A549 cell line				MG63 cell line			
	Gap of Day 0	Gap of Day 1	Gap of Day 2	Gap filling rate	Gap of Day 0	Gap of Day 1	Gap of Day 2	Gap filling rate
75	43.73	42.31	8.20	35.53	86.28	79.87	0	86.28
76	45.08	39.26	33.21	11.86	76.59	54,72	0	76.59
77	54.03	41.68	9.74	44.29	70,17	30.85	0	70,17
71	55.67	53.73	53.24	2.43	81.70	79.99	61.51	20.19
Control	51.56	17.82	0	51.56	89,83	19,27	0	89,83

When we increased the incubation time during this test, it was understood that while the control group continued to grow in a row, unique to cancer cells, the spaces in the insert not filled in the groups where the compounds 71-77 were applied. According to this test result, since these molecules inhibit cell migration, it is thought that this effect of (-)-camphene molecules is partly responsible for their anticancer activities.

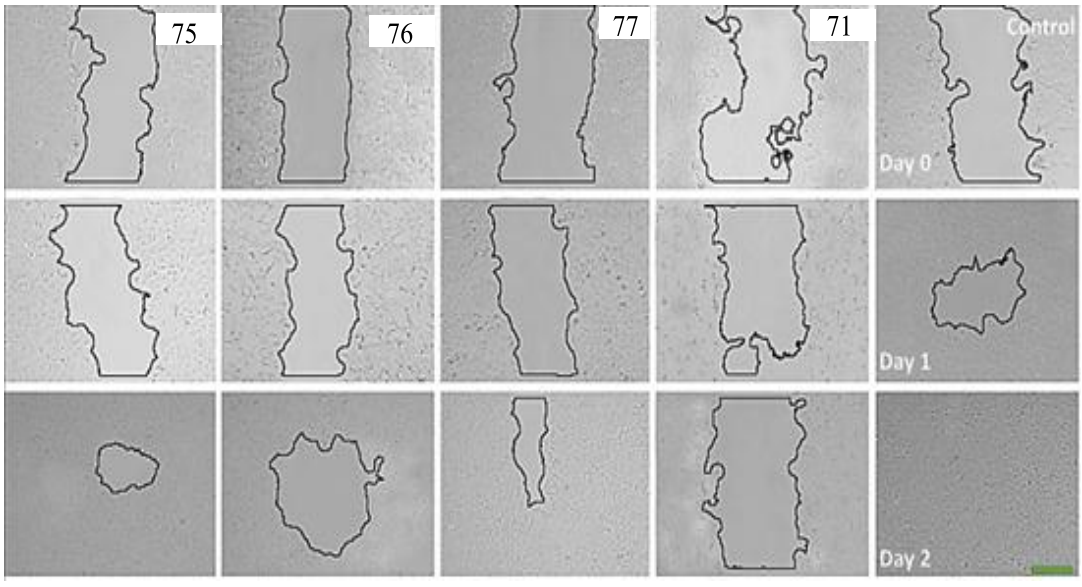


Figure 4.33. Effects of these compounds on cell migration on A549 cell lines. All scales are 100 μm.

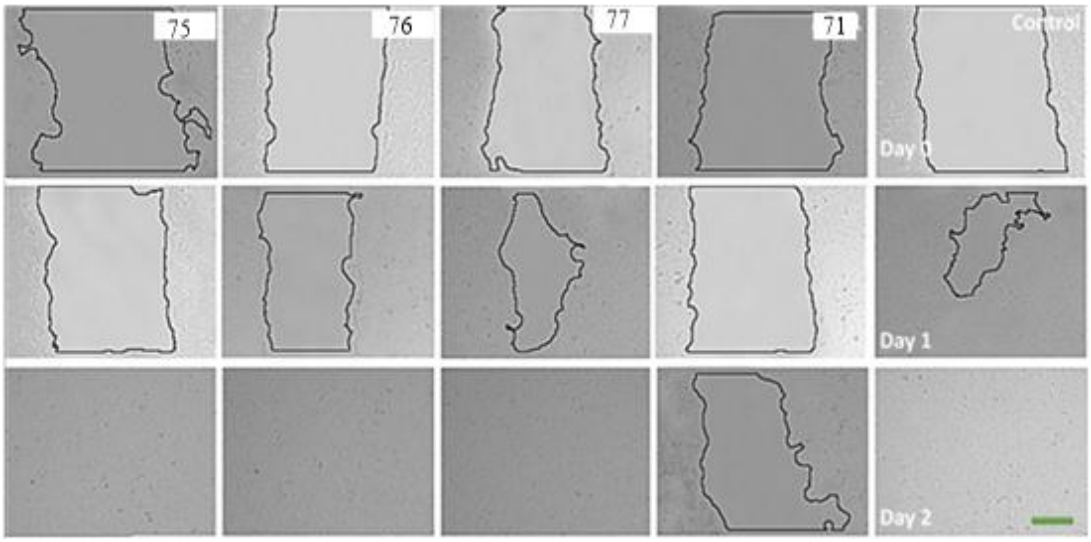


Figure 4.34. Effects of these compounds on cell migration on MG63 cell lines. All scales are 100 μm.

4.9.5. Effect of the new molecules on cell morphology

In this study, the morphological effects of the (-)-camphene molecules on lung cancer cells (A549, Calu1, and H1650) and bone cancer cells (MG63, Saos2, and SW1353), and normal lung cells (Beas2B), normal chondrocyte cells (HC) were observed and photographed 24 hours after application. Observed morphological alteration images here were distinguished in all cell lines treated with compounds 71, 73, and 77 for lung cells and compounds 74, 75, and 76 for bone cells. Some of them like weak cell attachment or floating cells, cell aggregation, cell rounding, granular formation, cell blebbing, cellular shrinkage, and disintegration of cell clumps were determined in the majority of the cells (Figures 4.35 and 4.36). The changes that occurred in the cell morphology by applying TGI doses of (-)-camphene molecules indicate that the effect mechanism of the (-)-camphene including apoptosis is in line with the MTT, DNA degradation, and cell migration test results of the (-)-camphene molecules.

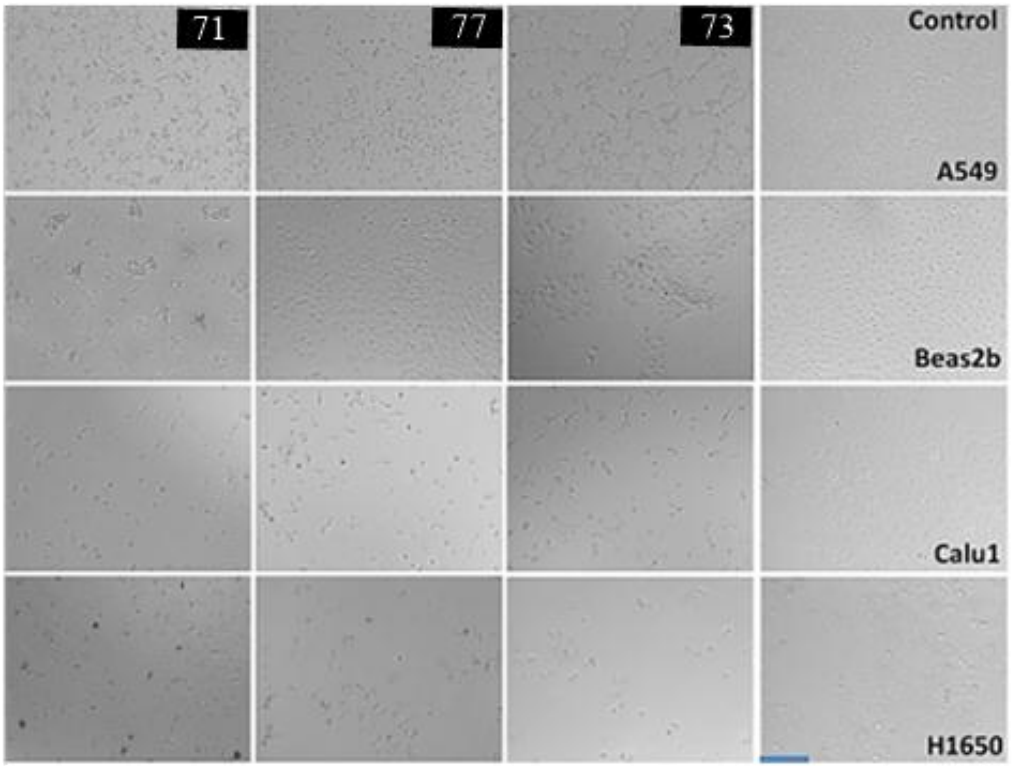


Figure 4.35. Effect of these molecules on the morphology of A549, Beas2B, Calu1, and H1650 cell lines. DMSO-treated cells as controls. All scale bars show 100 µm.

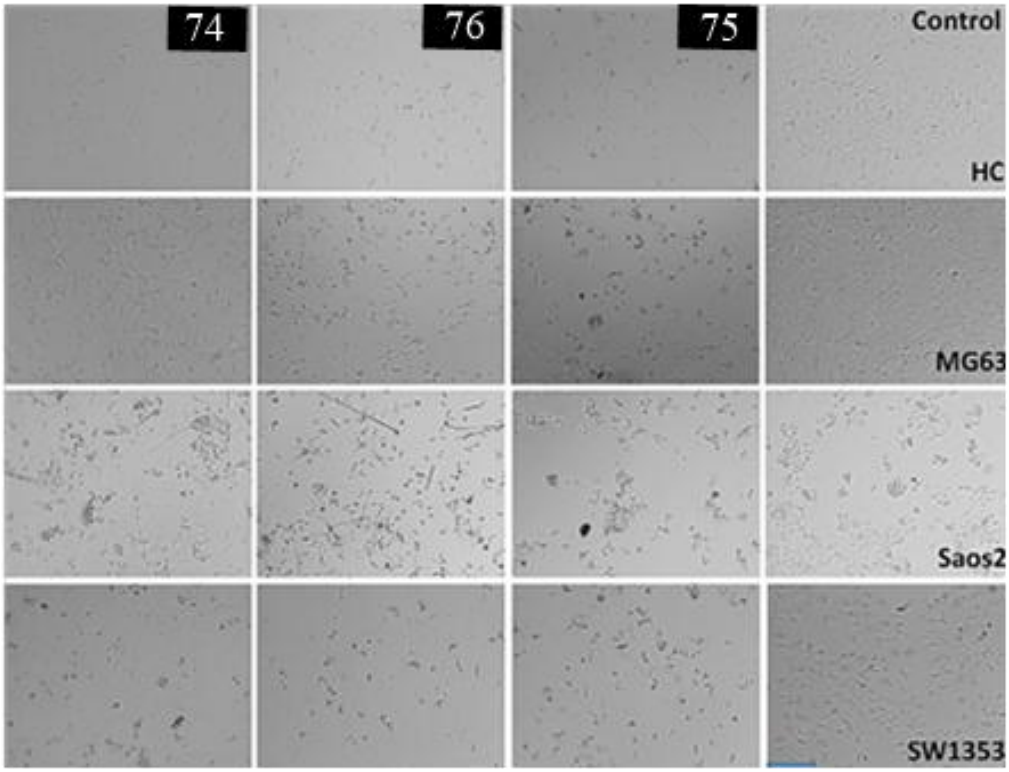


Figure 4.36. Effect of these molecules on the morphology of HC, MG63, Saos2, and SW1353 cell lines. DMSO-treated cells as controls. All scale bars show 100 µm.

4.9.6. Assessment of antibacterial and antifungal effects of the new (-)-camphene derivatives

In this study used the Minimum Inhibition Concentration (MIC) method to assessment the effects of compounds 73-77 on some pathogenic bacteria in the human body. The MIC values of today's antimicrobial drugs as a base we considered our test molecules to be antibacterial (256 μg/mL), and below MIC values. When MIC values of compounds 71-77 displayed on Gram (+), and Gram (-) bacteria were examined, it was found that the antibacterial effects of these molecules against to all strains (512 - > 512 μg/mL) were ineffective compared to SCF antibiotic used as a positive control (Table 4.6). Depending on MIC values exhibited by the compounds 71-77 on fungi it was determined all of the compounds against *C. albicans* ATCC 10231 (>512 μg/mL) strain were ineffective compared to FLZ antifungal used as positive control (Table 4.6). Therefore, these molecules lack antimicrobial properties against all bacteria and fungi.

Table 4.6. Minimum-inhibitory concentrations (MIC, in μg/mL) of compounds.

Microorganisms		74	76	75	71	77	73	72	**Control**
E. faecalis **VRE**	ATCC 19433	>512	>512	>512	>512	>512	>512	>512	8
E. faecalis	ATCC 29212	>512	>512	>512	>512	>512	>512	512	4
S. aureus	ATCC 25923	512	512	>512	512	>512	>512	>512	4
S. aureus **MSSA**	ATCC 29213	>512	>512	>512	>512	>512	>512	>512	8
S. aureus **MRSA**	ATCC 46300	>512	>512	>512	>512	>512	>512	>512	8
E. coli	ATCC 25922	>512	>512	>512	>512	>512	>512	512	4
P. aeruginosa **AGME**	ATCC 27853	>512	>512	>512	512	>512	>512	>512	8
S. gordonii	NCTC 7870	>512	>512	>512	>512	>512	>512	>512	4
A. actinomycetemcomitans	ATCC 33384	>512	>512	>512	>512	>512	>512	512	4
C. albicans	ATCC 10231	>512	>512	>512	>512	>512	>512	>512	64

*S/CF (sulbactam/cefoperazone) and FLZ (fluconazole) are positive control for bacteria and fungi, respectively.

5. CONCLUSION AND RECOMMENDATIONS

Our compounds have industrial, cosmetic, and therapeutic purposes. They are also employed in fragrance. It can be utilized to create further (-)-camphene. The substances we synthesized also served as a future starting point for the creation of other terpene derivatives that were used as study materials. Bromocamphene compounds can also be used in the synthesis of (-)-camphene derivatives 73-77. Pesticides, polymers, medicinal chemicals, and cosmetics are just a few examples of the many industrial uses for these substances. These bromo-(-)-camphene compounds serve as helpful reagents in the synthesis of several chemicals with important biological and pharmacological properties. Given that they are included in the structure of many physiologically active chemicals, products (-)-camphene is crucial. The usage of bromo-(-)-camphene is the most acceptable approach, even though other lengthy synthesis pathways have been documented. Compounds from our investigation that may have crucial structural components for the sources of the items we obtained were developed by derivatives that could be synthesized, and we tried to derivatives synthesize the structural activity of these substances. Our research's objective is to create (-)-camphene molecules' brominated derivatives, which will enable the creation of bioactive chemicals. In our study, firstly, the technological caliber 75% [90% as (-)-camphene and fenchene (70)] direct bromination reactions at low temperatures were carried out. Bromine was connected to the aliphatic ring using CH_2Cl_2, which we used as the bromine solution because of our selectivity tests, and 2,10-dibromo-(-)-camphene (71) molecules were produced with a high yield of 85% Thus, in a high-temperature elution reaction with aniline, 10-bromo-(-)-camphene (72) was created. A mixture of bioactive chemicals is produced when (72) combines with boronic acid compounds 73-77 according to the test results (Figure 5.1).

In the NCI-60 screening methodology, the findings demonstrated that 47 and 72 (GI_{50} value 1.07-1.68, TGI value 2.36-55.97, and LC_{50} value > 500 μg/mL for A549, Calu1, and H1650 lung cancer cells) were capable to induce an anticancer effect without affecting normal cells (GI_{50} value 1.16-1.27, TGI value 2.66-23.72, and LC_{50} value > 500 μg/mL for Beas2B normal lung cells), when considering all treated bone cancer

cell lines, the compounds 76 and 72 with strong anticancer effect (GI_{50} value 1.02-1.63, TGI value 1.22-33.47, and LC_{50} value 6.10 - > 500 μg/mL for MG63, Saos2, and SW1353 bone cancer cells) is the most convenient one for further preclinical studies because they exhibit low cytotoxicity towards normal chondrocyte HC cells (GI_{50} value 1.06-2.14, TGI value 2.26-39.79, and LC_{50} value > 500 μg/mL), 71 was very toxic against Beas 2B normal lung cells which precluded the use of it. When evaluating compounds 71, 72, and 76 together with MG63 bone cancer cells, it was expressed that these molecules exhibited strong antiproliferative effects When considering Saos2 cancer cells, these molecules exhibited similar or better anticancer effects than the control drug. 5FU, when examining Table 3, it was seen that the anticancer activity exhibited by subjected molecules except for compounds 73 and 77 was found to be at a similar or higher level compared to the anticancer medication 5FU in control in SW1353 cancer cells, when in contrast to the positive control 5FU, it was seen the compound 74 was very toxic against HC-normal chondrocyte cells (LC_{50} value 313.73 μg/mL), despite being found to be more affected on bone cancer cells. This situation limits the use of the compound's 74 agents. If these 71 and 74 molecules' toxic effects against lung and chondrocyte normal cells, respectively, are reduced by chemical modification, they may be a good option for cancer treatment.

When the findings from both normal and cancer cells were reviewed in detail, it was vigorously speculated that compound 72 is more cancer-specific for bone and lung cancer types, and that make it the most convenient one for further preclinical studies. Compound 72 at TGI concentration that were founded to be effective in MTT proliferation test showed similar cytotoxicity values of 17-19% for cancer and normal cell lines compared to the 5FU control drug. In addition, the other effective molecules, compound 74 for Beas2B lung normal cells (18.0) and 76 for HC chondrocyte normal cells (19.0), exhibited acceptable toxicity.

The result revealed of DNA laddering test for new (-)-camphene derivatives 71-77 induced the formation of DNA fragments in treated MG63, A549, and Beas2B cells compared to the untreated cells. That's more anticancer effects on cancer cells than normal cells. Therefore, one of the aims of newly developed (-)-camphene molecules is to significantly reduce the migration capacity of cancer cells. Migration test results of compounds 71, 75, 76, and 77 were found to be effective during the study, but after that, it turns out that this effect of (-)-camphene molecules is partly responsible for

their anticancer activities. The changes that occurred in the cell morphology by applying TGI doses of (-)-camphene molecules indicate that the effect mechanism of the (-)-camphene, including apoptosis, is in line with the MTT, DNA degradation, and cell migration test results of the (-)-camphene molecules. According to MIC values exhibited by the newly synthesized compounds of (-)-camphene on fungi, that was determined all of the compounds against the *C. albicans* ATCC 10231 (>512 µg/mL) strain were ineffective compared to the FLZ antifungal used as a positive control. Therefore, these molecules lack antimicrobial properties against all bacteria and fungi.

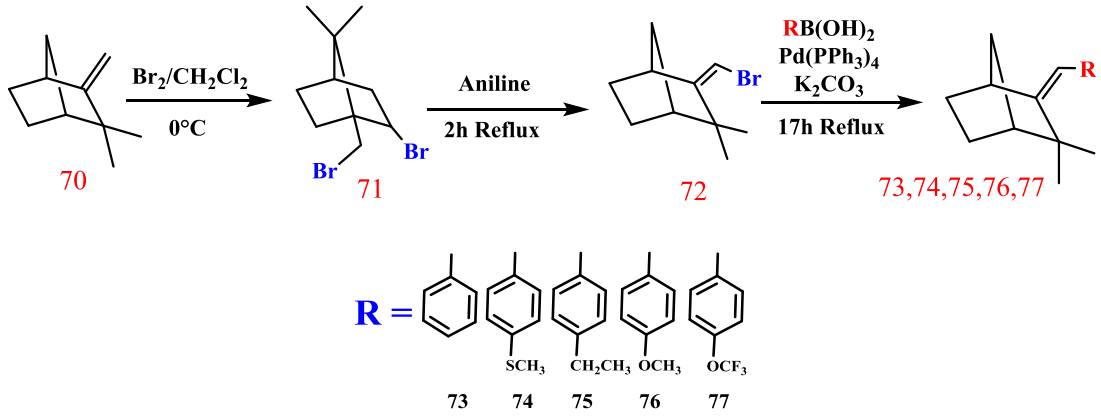


Figure 5.1. New (-)-camphene derivatives reactions.

REFERENCES

Acer, Ö. (2009). *Kamfenin yüksek sıcaklıkta fotobrominasyonu* [Doctoral dissertation]. Sakarya Universitesi (Türkiye).

Adamczyk-Woźniak, A., Gozdalik, J. T., Kaczorowska, E., Durka, K., Wieczorek, D., Zarzeczańska, D., & Sporzyński, A. (2021). (Trifluoromethoxy) phenylboronic acids: structures, properties, and antibacterial activity. *Molecules*, *26*(7), 2007. https://doi.org/10.3390/molecules26072007

Adorjan, B., & Buchbauer, G. (2010). Biological properties of essential oils: an updated review. *Flavour and Fragrance Journal*, *25*(6), 407-426. https://doi.org/10.1002/ffj.2024

Baek, S., Kim, J., Moon, B. S., Park, S. M., Jung, D. E., Kang, S. Y., ... & Lee, K. P. (2020). Camphene attenuates skeletal muscle atrophy by regulating oxidative stress and lipid metabolism in rats. *Nutrients*, *12*(12), 3731. https://doi.org/10.3390/nu12123731

Balkancı, A. (2010). *α-pinen'in yüksek sıcaklık fotobraminasyonu ve bazı α-pinenlerin sentezi* [Doctoral dissertation] Sakarya Universitesi (Türkiye).

Banthorpe, D. V., & Baxendale, D. (1968). The biosynthesis (+)-and (–)-camphor. *Chemical Communications (London)*, (23), 1553-1554. https://doi.org/10.1039/C19680001553

Birladeanu, L. (2000). The story of the Wagner-Meerwein rearrangement. *Journal of Chemical Education*, *77*(7), 858. https://doi.org/10.1021/ed077p858

Brown, R. S. (1997). Investigation of the early steps in electrophilic bromination through the study of the reaction with sterically encumbered olefins. *Accounts of Chemical Research*, *30*(3), 131-137. https://doi.org/10.1021/ar960088e

Ceylan, A., Medicinal Plants II, Ege University Faculty of Agriculture Publication. 481, 1-22, 1987.

Chen, N., Yang, P., He, X., Shao, M., & Li, M. X. (2013). Synthesis, structure, luminescence, and magnetic property of camphorate coordination polymers containing 5, 6-dimethylbenzimidazole auxiliary ligand. *Inorganica Chimica Acta*, *405*, 43-50. https://doi.org/10.1016/j.ica.2013.05.010

Christianson, D. W. (2017). Structural and chemical biology of terpenoid cyclases. *Chemical Reviews*, *117*(17), 11570-11648. https://doi.org/10.1021/acs.chemrev.7b00287

Chukicheva, I. Y., Buravlev, E. V., Fedorova, I. V., Borisenkov, M. F., & Kutchin, A. V. (2010). Antioxidant properties of terpene-substituted phenols. *Russian Chemical Bulletin*, *59*, 2276-2280. https://doi.org/10.1007/s11172-010-0388-5

Collins, C. J., & Harding, C. E. (1969). Ratio of the rates of solvent attack and 3, 2-hydride shift in the norbornyl cation. *Journal of the American Chemical Society*, *91*(25), 7194-7195. https://doi.org/10.1021/ja01053a053

Croteau, R., Satterwhite, D. M., Cane, D. E., & Chang, C. C. (1988). Biosynthesis of monoterpenes. Enantioselectivity in the enzymatic cyclization of (+)-and (-)-linalyl pyrophosphate to (+)-and (-)-pinene and (+)-and (-)-camphene. *Journal of Biological Chemistry*, *263*(21), 10063-10071. https://doi.org/10.1016/S0021-9258(19)81477-1

De Freitas, B. C., Queiroz, P. A., Baldin, V. P., do Amaral, P. H., Rodrigues, L. L., Vandresen, F., ... & Siqueira, V. L. (2020). (-)-Camphene-based derivatives as potential antibacterial agents against *Staphylococcus aureus* and *Enterococcus spp*. *Future Microbiology*, *15*(16), 1527-1534. https://doi.org/10.2217/fmb-2020-0131

De la Mare, P. B. D., & Bolton, R. (2013). *Electrophilic additions to unsaturated systems*. Elsevier.

Decker, T. & Lohmann-Matthes, M. L. (1988) A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. *Journal of Immunological Methods*, *115*(1), 61-69. https://doi.org/10.1016/0022-1759(88)90310-9

Demirci Gültekin, D. (2005). *Norbornen ve türevlerinin değişik sıcaklıklarda brominasyonu: Polibromlu norbornan ve norbornenlerin sentezi* [Doctoral dissertation] Erzurum.

Foca, C. M., dos Santos, E. N., & Gusevskaya, E. V. (2002). Diastereoselective hydroformylation of camphene catalyzed by platinum/tin complexes. *Journal of Molecular Catalysis A: Chemical*, *185*(1-2), 17-23. https://doi.org/10.1016/S1381-1169(02)00056-0

Garamszegi, L., & Schlosser, M. (1998). An unprecedented elimination- driven migration: the reductive dihalobornane–camphene rearrangement. *Angewandte Chemie International Edition*, *37*(22), 3173-3175. https://doi.org/10.1002/(SICI)1521-3773(19981204)37:22%3C3173::AID-ANIE3173%3E3.0.CO;2-Y

Girola, N., Figueiredo, C. R., Farias, C. F., Azevedo, R. A., Ferreira, A. K., Teixeira, S. F., ... & Lago, J. H. (2015). Camphene isolated from the essential oil of Piper cernuum (Piperaceae) induces intrinsic apoptosis in melanoma cells and displays antitumor activity in vivo. *Biochemical and Biophysical Research Communications*, *467*(4), 928-934. https://doi.org/10.1016/j.bbrc.2015.10.041

Gong, J. P., Traganos, F., Darzynkiewicz, Z. (1994) A selective procedure for DNA extraction from apoptotic cells applicable for gel electrophoresis and flow cytometry. *Analytical Biochemistry*, *218*(2), 314-319. https://doi.org/10.1006/abio.1994.1184

Gujral, S. S., Khatri, S., Riyal, P., & Gahlot, V. (2012). Suzuki cross-coupling reaction review. *Indo Global Journal of Pharmaceutical Sciences, 2(4), 351-367*.

Gültekin, D. D., Taşkesenligil, Y., Daştan, A., & Balci, M. (2008). Bromination of norbornene derivatives: Synthesis of brominated norbornanes and norbornenes. *Tetrahedron*, *64*(19), 4377-4383. https://doi.org/10.1016/j.tet.2008.02.067

Hachlafi, N. E., Aanniz, T., Menyiy, N. E., Baaboua, A. E., Omari, N. E., Balahbib, A., ... & Bouyahya, A. (2023). In vitro and in vivo biological investigations of camphene and its mechanism insights: A review. *Food Reviews International*, *39*(4), 1799-1826. https://doi.org/10.1080/87559129.2021.1936007

Harborne, J. B., Baxter, H., & Moss, G. P. A. (1993). A handbook of bioactive compounds from plants. *Phytochemical Dictionary*, *35*, 36-37.

Horasan, N., Kara, Y., Azizoğlu, A., & Balci, M. (2003). Low and high-temperature bromination of exocyclic dienes: high-temperature bromination. Part 16. *Tetrahedron*, *59*(21), 3691-3699. https://doi.org/10.1016/S0040-4020(03)00549-0

Hossain, M. A., Ismail, Z., Rahman, A., & Kang, S. C. (2008). Chemical composition and anti-fungal properties of the essential oils and crude extracts of *Orthosiphon stamineus* Benth. *Industrial Crops and Products*, *27*(3), 328-334. https://doi.org/10.1016/j.indcrop.2007.11.008

Khaleel, C., Tabanca, N., & Buchbauer, G. (2018). α-Terpineol, a natural monoterpene: A review of its biological properties. *Open Chemistry*, *16*(1), 349-361. https://doi.org/10.1515/chem-2018-0040

Kim J. H. (2018). A volatile terpene component contained in the root of the Atractylodes genus plant. Journal of *Oriental Medicine Information Research*, 6(1), 1-17. https://doi.org/10.22674/KHMI-6-1-1

Lana, E. J. L., da Silva Rocha, K. A., Kozhevnikov, I. V., & Gusevskaya, E. V. (2006). One-pot synthesis of diisobornyl ether from camphene using heteropoly acid catalysts. *Journal of Molecular Catalysis A: Chemical*, *243*(2), 258-263. https://doi.org/10.1016/j.molcata.2005.08.038

Liu, C., Zhang, H., Shi, W., & Lei, A. (2011). Bond formations between two nucleophiles: transition metal catalyzed oxidative cross-coupling reactions. *Chemical Reviews*, *111*(3), 1780-1824. https://doi.org/10.1021/cr100379j

Mabou, F. D., & Yossa, I. B. N. (2021). Terpenes: Structural classification and biological activities. *IOSR Journal of Pharmacy and Biological Sciences*, *16*(3), 25-40. https://doi.org/10.9790/3008-1603012540

Machado, T. Q., da Fonseca, A. C., Duarte, A., Robbs, B. K., & de Sousa, D. P. (2022). A narrative review of the antitumor activity of monoterpenes from essential oils: An update. *BioMed Research International*, *2022*. https://doi.org/10.1155/2022/6317201

Martinez, A. L., Gonzalez-Trujano, M. E., Chávez, M., & Pellicer, F. (2012). Antinociceptive effectiveness of triterpenes from rosemary in visceral nociception. *Journal of Ethnopharmacology*, *142*(1), 28-34. https://doi.org/10.1016/j.jep.2012.03.052

Meinwald, J., & Wiley, G. A. (1958). The synthesis and rearrangement of some benzonorbornenes[1a,1b]. *Journal of the American Chemical Society*, *80*(14), 3667-3671. https://doi.org/10.1021/ja01547a049

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*. *65*(1–2), 55-63. doi:10.1016/0022-1759(83)90303-4

Mort, B. C., & Autschbach, J. (2007). Temperature dependence of the optical rotation in six bicyclic organic molecules calculated by vibrational averaging. *ChemPhysChem*, *8*(4), 605-616. https://doi.org/10.1002/cphc.200600757

Nevell, T. P., De Salas, E., & Wilson, C. L. (1939). 259. Use of isotopes in chemical reactions. Part I. The mechanism of the Wagner–Meerwein rearrangement. Exchange of radioactive chlorine and deuterium between camphene hydrochloride and hydrogen chloride. *Journal of the Chemical Society (Resumed)*, 1188-1199. https://doi.org/10.1039/JR9390001188

Nikitina, L. E., Artemova, N. P., Startseva, V. A., Fedyunina, I. V., & Klochkov, V. V. (2017). Biological activity of S-containing monoterpenoids. *Chemistry of Natural Compounds*, *53*, 811-819. https://doi.org/10.1007/s10600-017-2131-z

Perrin, D. D., Armarego, W. L., & Perrin, D. R. (1988). Purification of laboratory chemicals.

Prates, H. T., Santos, J. P., Waquil, J. M., Fabris, J. D., Oliveira, A. B., & Foster, J. E. (1998). Insecticidal activity of monoterpenes against *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst). *Journal of Stored Products Research*, *34*(4), 243-249. https://doi.org/10.1016/S0022-474X(98)00005-8

Quintans-Júnior, L., Moreira, J. C., Pasquali, M. A., Rabie, S., Pires, A. S., Schröder, R., ... & Gelain, D. P. (2013). Antinociceptive activity and redox profile of the monoterpenes. *International Scholarly Research Notices*, *2013*. http://dx.doi.org/10.1155/2013/459530

Ramos da Silva, L. R., Ferreira, O. O., Cruz, J. N., de Jesus Pereira Franco, C., Oliveira dos Anjos, T., Cascaes, M. M., ... & Santana de Oliveira, M. (2021). Lamiaceae essential oils, phytochemical profile, antioxidant, and biological activities. *Evidence-Based Complementary and Alternative Medicine*, *2021*. https://doi.org/10.1155/2021/6748052

Rasoul, M. A. A., Marei, G. I. K., & Abdelgaleil, S. A. (2012). Evaluation of antibacterial properties and biochemical effects of monoterpenes on plant pathogenic bacteria. *African Journal of Microbiology Research*, *6*(15), 3667-3672. https://doi.org/10.5897/AJMR12.118

Roglans, A., Pla-Quintana, A., & Moreno-Manas, M. (2006). Diazonium salts as substrates in palladium-catalyzed cross-coupling reactions. *Chemical Reviews*, *106*(11), 4622-4643. https://doi.org/10.1021/cr0509861

Smith, W. B. (1999). A DFT study of the camphene hydrochloride rearrangement. *The Journal of Organic Chemistry*, *64*(1), 60-64. https://doi.org/10.1021/jo980720c

Sokolova, A. S., Putilova, V. P., Yarovaya, O. I., Zybkina, A. V., Mordvinova, E. D., Zaykovskaya, A. V., ... & Salakhutdinov, N. F. (2021). Synthesis and antiviral activity of camphene derivatives against different types of viruses. *Molecules*, *26*(8), 2235. https://doi.org/10.3390/molecules26082235

Sonawane, H. R., Nanjundiah, B. S., & Rajput, S. I. (1984). Photochemistry of vinyl halides. 1. Radical versus ionic photobehavior of some vinyl halides based on camphene. *Indian Journal of Chemistry Section B-Organic Chemistry Including Medicinal Chemistry*, *23*(4), 331-338.

Souza, M. R., Coelho, N. P., Baldin, V. P., Scodro, R. B., Cardoso, R. F., da Silva, C. C., & Vandresen, F. (2019). Synthesis of novel (-)-camphene-based thiosemicarbazones and evaluation of anti-Mycobacterium tuberculosis activity. *Natural Product Research*, *33*(23), 3372-3377. https://doi.org/10.1080/14786419.2018.1478829

Thurman, E. M. (2020). Analysis of terpenes in hemp (*Cannabis sativa*) by gas chromatography/mass spectrometry: Isomer identification analysis. *Comprehensive Analytical Chemistry*, *90*(2020), 197-233. https://doi.org/10.1016/bs.coac.2020.04.013

Titova, T. F., Korchagina, D. V., & Barkhash, V. A. (1995). Bromination of camphene. *ChemInform*, *26*(51), 83-85.

Trombetta, D., Castelli, F., Sarpietro, M. G., Venuti, V., Cristani, M., Daniele, C., ... & Bisignano, G. (2005). Mechanisms of antibacterial action of three monoterpenes. *Antimicrobial Agents and Chemotherapy*, *49*(6), 2474-2478. https://doi.org/10.1128/aac.49.6.2474-2478.2005

Tutar, A., & Balci, M. (2002). Bromination of an N-carbethoxy-7-aza-2, 3-benzonorbornadiene, and synthesis of N-carbethoxy-7-aza-2, 3-dibromo-5, 6-benzonorbornadiene: high temperature bromination. Part 14. *Tetrahedron*, *58*(44), 8979-8984. https://doi.org/10.1016/S0040-4020(02)01150-X

Tutar, A. (1999). *Some uses of organobromine compounds* [Doctoral dissertation] Tokat Gaziosmanpaşa Universitesi (Türkiye).

Tutar, A., Taskesenligil, Y., Abbasoglu, R., & Balci, M. (1996). High temperature bromination. 7.(1) bromination of norbornadiene. *The Journal of Organic Chemistry*, *61*(23), 8297-8300. https://doi.org/10.1021/jo960225l

Vallianou, I., Peroulis, N., Pantazis, P., & Hadzopoulou-Cladaras, M. (2011). Camphene, a plant-derived monoterpene, reduces plasma cholesterol and triglycerides in hyperlipidemic rats independently of HMG-CoA reductase activity. *PloS One*, *6*(11), e20516.

https://doi.org/10.1371/journal.pone.0020516

Wang, H., Ma, D., Yang, J., Deng, K., Li, M., Ji, X., ... & Zhao, H. (2018). An integrative volatile terpenoid profiling and transcriptomics analysis for gene mining and functional characterization of AvBPPS and AvPS involved in the monoterpenoid biosynthesis in *Amomum villosum*. *Frontiers in Plant Science*, *9*, 846. https://doi.org/10.3389/fpls.2018.00846

Wiberg, K. B., Wang, Y. G., Murphy, M. J., & Vaccaro, P. H. (2004). Temperature dependence of optical rotation: α-pinene, β-pinene pinene, camphene, camphor, and fenchone. *The Journal of Physical Chemistry A*, *108*(26), 5559-5563. https://doi.org/10.1021/jp040085g

Winstein, S. (1961). Bicycloheptadiene dibromides. *Journal of the American Chemical Society*, *83*(6), 1516-1517. https://doi.org/10.1021/ja01467a058

Winstein, S., & Trifan, D. (1952). Neighboring carbon and hydrogen. X. solvolysis of endo-norbornyl arylsulfonates[1,2,3]. *Journal of the American Chemical Society*, *74*(5), 1147-1154. https://doi.org/10.1021/ja01125a006

Yılmaz, R. F., Derin, Y., Mısır Albayrak, B., Tutar, O. F., Atalay, E. V., Okten, S., Tutar, A. (2023). Spectral properties of symmetrically arylated BODIPY dyes: Experimental and computational approach, *Journal of Molecular Structure*, 135962, 2023. https://doi.org/10.1016/j.molstruc.2023.135962

Yılmaz, R.F., Islam, M. Z., Ökten, S., Şahin, E., Daştan, A. and Tutar, A. (Unpublished). Photobromination of (+)-camphene and synthesis of novel compounds from monobrominated (+)-camphene based derivatives.

APPENDICES

APPENDIX A. Figures

APPENDIX A

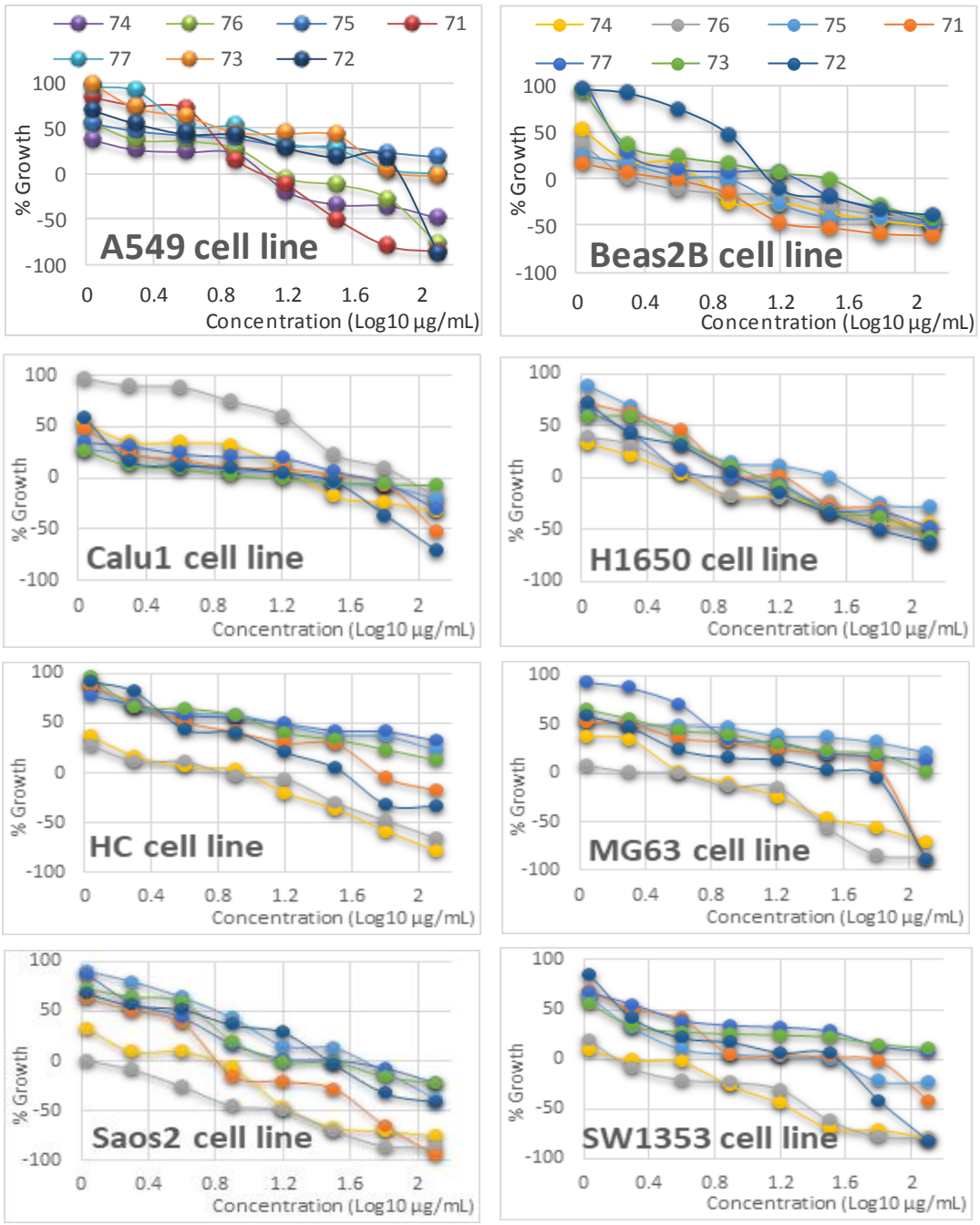


Figure A.1. Antiproliferative activity of these compounds on cell lines. Cell proliferation was measured using an MTT assay.

CURRICULUM VITAE

Name Surname: Ansam ALMOTORY

EDUCATION:

Undergraduate: 2003 B.D, Basra University, Chemistry Department.

Graduate : 2023 Master Sakarya University, Graduate School of Natural and Applied Sciences, Chemistry Department.

PROFESSIONAL EXPERIENCE AND AWARDS:

- She worked as a line manager at the Iraqi-American humanitarian organizations for humans between 2004-2006.
- She worked as a line manager at Basra Laboratories Central of WWT between 2007-2012.
- She worked as a senior project manager of the Basra WWT Directorate since 2012.

PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:

- Almotory, A., Derin, Y., Albayrak Mısır, B., Tutar, A. (2023, 05-06, May). Synthesis of new (-)-camphene derivatives with Suzuki coupling reactions. 2nd International scientific conference on the "Actual Problems of the Natural and Economic Sciences" dedicated to the 100th anniversary of National Leader Haydar Aliyev. p.175, Ganja State University, Ganja, Azerbaijan.