UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE FARMÁCIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

PLANEJAMENTO E SÍNTESE DE NOVOS COMPOSTOS VISANDO ATIVIDADE BIOLÓGICA

CESAR EMILIANO HOFFMANN DA SILVA

Porto Alegre, 2020

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Dissertação apresentada por **Cesar Emiliano Hoffmann da Silva** para obtenção do GRAU DE MESTRE em Ciências Farmacêuticas.

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““…. Quando comecei a fazer química, fiz da maneira que pescava - pela emoção, pela descoberta, pela aventura, por ir atrás das capturas mais elusivas que se possa imaginar em mares desconhecidos.”. ”

—Karl Barry Sharpless

RESUMO

O interesse no estudo das propriedades biológicas de produtos naturais como os terpenos tem crescido nas últimas décadas, os quais têm sido utilizados em diversos processos etiológicos. Um dos interesses atuais é a busca de novos repelentes, originários de moléculas naturais. Explorando o potencial de pequenas moléculas orgânicas como esqueleto para a síntese de novos protótipos repelentes, a síntese de quatro séries de análogos da D-cânfora, eugenol, D-limoneno, e L-mentona foi realizada em conjunto com uma série adicional de compostos antranílicos, promissores repelentes, totalizando 23 derivados. Como estratégia sintética, para o eugenol e o D-Limoneno foi utilizada a dihidroxilação assimétrica de Sharpless para evitar a formação de misturas e selecionar os isômeros desejados. Todos os derivados obtidos foram caracterizados por ressonância magnética nuclear e espectrometria de massas, onde um derivado foi descrito completamente pela primeira vez na literatura. Foi realizada uma discussão sobre o mecanismo de alguns produtos obtidos, e comparação dos resultados com a literatura. Os derivados foram submetidos a testes preliminares *in vitro* como repelentes contra *Aedes aegypti* para prevenção da transmissão de arbovírus. Estes testes foram baseados no número de pousos e picadas, bem como o cálculo do índice de proteção de picadas, em uma concentração de 200 nmoles/cm^2. Dos resultados preliminares obtidos, cinco moléculas apresentaram resultados similares ao repelente comercial mais usado o DEET, sendo promissores candidatos a se tornarem repelentes comerciais. Também foi proposto um perfil de estrutura atividade, bem como as perspectivas futuras em relação a este trabalho. Em um capítulo adicional, o limoneno e o seu metabólito natural, álcool perílico, foram escolhidos para uma completa revisão sistemática da literatura baseado em seus derivados sintéticos frente a atividade antitumoral.

Palavras-Chave: *Aedes aegypti*, arbovírus, repelentes, limoneno, mentona, cânfora, eugenol, síntese assimétrica, prevenção, DEET, mosquitos

ABSTRACT

The interest in the study of the biological properties of natural products such as terpenes has grown in recent decades, which compounds have been used in various etiological processes. One of the current interests is the search for new repellents from natural molecules. Exploring the potential of small organic molecules as scaffold for the synthesis of new repellent prototypes, the synthesis of four series of D-camphor, eugenol, D-limonene, L-menthone analogs was performed together with an additional series of anthranilic compounds, promising repellents, totaling 23 derivatives. As a synthetic strategy, for eugenol and D-Limonene, the Sharpless asymmetric dihydroxylation was used to avoid the formation of mixtures and to select the desired isomers. All derivatives obtained were characterized by nuclear magnetic resonance and mass spectrometry, where one derivative was completely described for the first time in the literature. It is presented a discussion about the mechanism of some products obtained, and a comparison of the results with literature. The derivatives were subjected to preliminary *in vitro* tests as repellents against *Aedes aegypti* to prevent arbovirus transmission. These tests were based on the number of landings and stings as well as the calculation of the sting protection index at a concentration of 200 nmol/cm^2. From the preliminary results, five molecules showed results similar to the most commonly used commercial repellent, DEET, being promising candidates to become commercial repellents. An activity structure profile was also proposed, as well as future perspectives regarding this work. In an additional chapter, limonene and its natural metabolite, perillyl alcohol, were chosen for a complete systematic review of the literature based on its synthetic derivatives against antitumor activity.

Keywords: *Aedes aegypti*, arbovirus, repellents, limonene, menthone, camphor, eugenol, asymmetric synthesis, prevention, DEET, mosquitoes

LISTA DE ABREVIATURAS E SIGLAS

BVO	Baeyer-Villiger Oxidation
CC	column chromatography
$CDCl_3$	Deuterated Chloroform
DEET	N,N-dimetil-meta-toluamida
DMAP	4-Dimethylaminopyridine
EtOH	Ethanol
Et_3N	Triethylamine
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
GC	Gas Chromatography
Hz	Hertz
K_2CO_3	Potassium Carbonate
$K_2OsO_2(OH)_4$	Potassium osmate(VI) dihydrate
$K_3Fe\ [CN_6]$	Potassium ferricyanide
m-CPBA	m-chloroperbenzoic acid
MS	mass spectrometer
NaOH	Sodium Hydroxide
$NaHCO_3$	Sodium Bicarbonate
Na_2SO_4	Sodium Sulfate
NMR	Nuclear Magnetic Resonance
Ppm	parts per million
qt.	Quaternary
QSAR	Quantitative Structure Activity Relationships
TfOH	Triflic acid
TLC	Thin Layer Chromatography
TsOH	Tosylic acid (p-toluene sulfonic acid)
td	Tripleto de dupletos
THF	Tetrahydrofuran
δ	Chemical shift

SUMÁRIO

INTRODUÇÃO **17**
OBJETIVOS **19**
REVISÃO BIBLIOGRÁFICA **21**
CAPÍTULO 1. Synthesis and structure-activity relationships of natural based promising repellents for *Aedes aegypti* **39**
1. INTRODUCTION 38
2. MATERIALS AND METHODS 41
3. RESULTS AND DISCUSSION 60
4. CONCLUSION 90
5. REFERENCES 91
6. SUPPLEMENTARY MATERIAL 99

CAPÍTULO 2. Synthesis and Anticancer activities and preliminary structure-activity relationships of Limonene and Perillyl alcohol Derivatives **151**
1. INTRODUCTION 151
2. MODIFICATIONS AT THE HYDROXYL GROUP 161
3. SYNTHETIC HYBRIDS WITH SPECIFIC CHEMICAL CLASSES 174
4. CONCLUSION 182

CONSIDERAÇÕES FINAIS 193

INTRODUÇÃO

A busca por novos compostos com atividades biológicas desejadas é um dos maiores objetivos da Síntese Orgânica Medicinal. Sendo uma ciência versátil, as possibilidades de exploração no campo da pesquisa, em especial, no campo de fármacos, torna-se essencial.

A construção de novas moléculas se dá por vias completamente sintéticas ou utilizando compostos naturais como esqueletos, que por intermédio da síntese orgânica, tem suas atividades refinadas e aprimoradas.

Com o interesse renovado nos últimos anos de utilizar fontes naturais para candidatos a fármacos, numerosos exemplos aparecem na literatura com resultados bastante promissores e práticos, justificando a importância da exploração e investimento em pesquisa nestes tópicos.

Um dos temas de interesse atual, utilizando de esqueletos naturais e usando a síntese como ferramenta, busca por finalidade a prevenção de enfermidades e epidemias que acometem países tropicais em desenvolvimento. Esta prevenção se dá pela síntese de novos candidatos a repelentes que possam ser mais eficazes que os produtos comerciais existentes, e que também se apresentem como uma alternativa advinda de fontes naturais. Além da prevenção, estes derivados também podem contornar possíveis problemas de resistência dos artrópodes expostos aos repelentes já existentes.

Outro tema, se configura na busca de novos terapêuticos para tratamento de câncer que possam ser mais eficazes aos existentes. Nas últimas duas décadas, o limoneno e seu metabólito natural, o álcool perílico, tem se destacado como potenciais agentes anticâncer, e aparecido em vários trabalhos envolvendo suas atividades intrínsecas. Por conta de suas estruturas passíveis de modificação, nos últimos vinte anos, vários estudos se utilizam da síntese orgânica para melhorar e refinar esta atividade. Essas informações valiosas encontram-se distribuídas de forma desordenada pela literatura, sendo por isso válida uma revisão sistemática.

Posto a importância destes assuntos, essa dissertação é organizada em dois capítulos, sendo em primeiro momento, um capítulo apresentando e discutindo a síntese e atividade de novas moléculas candidatas a repelentes. Neste capítulo encontra-se uma revisão bibliográfica atualizada e a caracterização completa desses

derivados, bem como uma discussão aprofundada sobre os mecanismos sintéticos e correlação entre estrutura e atividade.

No capítulo posterior, encontra-se a proposta de uma revisão sistemática de literatura para a atividade antitumoral de derivados sintéticos do limoneno e do álcool perílico, para publicação em um periódico de química medicinal.

OBJETIVOS

OBJETIVO PRINCIPAL. Planejar e sintetizar quatro séries de compostos terpênicos e uma série de derivados do ácido antranílico com atividade repelente para *Aedes aegypti in vitro*. Também, será realizado uma revisão sistemática sobre os derivados sintéticos do limoneno e do álcool perílico frente a atividade anticâncer.

Objetivos específicos

- Sintetizar 17 compostos em quatro séries de derivados baseados em: D-cânfora, eugenol, D-limoneno, L-mentona;
- Sintetizar 06 derivados do ácido antranílico
- Avaliar o efeito do tratamento dos derivados sintetizados sobre a ação repelente, de *Aedes aegypti*;
- Propor uma revisão sistemática dos derivados sintéticos do limoneno e do álcool perílico frente a atividade anticâncer, agrupando os resultados por ordem de similaridade e estratégias sintéticas adotadas.

REVISÃO BIBLIOGRÁFICA

Esta revisão bibliográfica foi realizada no período de 2000 a 2019 nas bases de dados Web of Science, PubMed, Scopus e Google Scholar utilizando as seguintes palavras-chaves: “zika”, “repellents”, “brazil”, “arboviruses” “epidemics”, “mosquitoes”, “repellency”, “arthropodes”, “synthetic repellents” “deet”.

Nos últimos anos, o reaparecimento de epidemias relacionadas aos arbovírus tem causado grande preocupação em relação à saúde pública em muitos países e sinalizado um alerta vermelho mundial. Os arbovírus podem ser transmitidos aos hospedeiros vertebrados por vetores tipo artrópodes hematófagos, sendo os mosquitos os mais destacados e bem conhecidos, em particular aqueles dos gêneros *Anopheles* e *Aedes*, responsáveis pela transmissão de doenças graves como a malária, a dengue e o Zika (FAUCI e MORENS, 2016; PETERSEN *et al*., 2016).

Os mosquitos *Aedes* spp estão presentes em todos os continentes, principalmente em regiões ao longo dos trópicos. Sua distribuição pode afetar a vida de mais de 3 bilhões de indivíduos que vivem nessas áreas infestadas. (WILDER-SMITH *et al*., 2017). O principal vetor associado à transmissão do vírus Zika é o mosquito *Aedes aegypti*. Esse vetor vive e se reproduz em ambientes domésticos, depositando seus ovos em locais que acumulam água parada. A transmissão ocorre através da picada do mosquito, principalmente durante o dia (PETERSEN *et al*., 2016).

O vírus zika (ZIKV) é um arbovírus emergente, isolado pela primeira vez do sangue de um macaco Rhesus em 1947, em Uganda. Em 2014, o vírus foi espalhado entre diversos países da Oceania. A epidemia continuou a se expandir rapidamente e numerosos casos foram relatados em países da América Latina (MUSSO *et al*., 2015). A partir de 2017, os casos de Zika tem declinado em números totais, mas ainda, um novo número de casos é reportado em países que não foram afetados pelo surto inicial (FERRARIS *et al.*, 2019).

O Brasil se destacou como o país latino-americano mais atingido, com casos estimados de infecção pelo vírus zika de 500.000 a 1.500.000, sendo o primeiro caso relatado em março de 2015. Com base na relação filogenética semelhante entre cepas encontradas na América do Sul, Ásia e Oceania, o vírus pode ter sido transmitido no país por viajantes asiáticos ou oceânicos durante os principais eventos esportivos que ocorreram no Brasil em 2014. (PETERSEN *et al*., 2016)

As manifestações clínicas relacionadas às infecções por ZIKV não são específicas (febre leve, exantema, artralgia e conjuntivite) e podem ser confundidas com outras doenças, principalmente dengue e chikunguya. No Brasil, o Ministério da Saúde relatou um aumento anormal no número de casos de recém-nascidos com microcefalia na região nordeste do país em outubro de 2015, coincidindo com a rápida expansão da epidemia de zika. Mais de 3000 casos suspeitos, incluindo casos fatais, foram relatados apenas no segundo semestre de 2015. Isso representa um aumento de 20 vezes nos relatos de microcefalia em comparação com os anos anteriores. A seriedade da situação levou a Organização Mundial da Saúde (OMS) a declarar a epidemia como uma “emergência global” (MUSSO *et al*., 2015; CHAN *et al*., 2016; PETERSEN *et al*., 2016).

Em relação ao nosso país, devido ao Ministério da Saúde declarar estado de emergência em novembro de 2015, e um esforço conjunto de profissionais da saúde, os números de casos foram caindo ano após ano. Em 2017, 13.353 novos casos foram reportados, um número bem abaixo do encontrado em 2016 (205.578), sendo que o principal responsável por esse decaimento é a imunização da população. Apesar disso, o virus Zika ainda continuará a circular pela população brasileira, bem como outras enfermidades relacionadas a epidemia de mosquito, como a dengue. (LOWE *et al.*, 2018)

Medidas de combate e prevenção

Considerando que o combate ao vetor ainda seja ineficiente em muitos países bem como o crescimento da resistência a inseticidas (DUSFOUR *et al.*, 2019), e a falta de vacinas contra o vírus Zika, recomenda-se o uso de medidas preventivas, como evitar a exposição ao mosquito durante o dia e proteção pessoal com o uso de repelentes. Essas medidas são especialmente importantes para gestantes, com base na associação entre infecção por vírus e casos de microcefalia de recém-nascidos. (BOGOCH *et al*., 2016).

Os repelentes tópicos são ferramentas importantes para prevenir infecções de agentes etiológicos transmitidos por vetores e são considerados a primeira linha de defesa contra insetos transmissores. Várias vantagens podem ser enumeradas para o uso de repelentes: são baratas, práticas e têm uma aplicação fácil de proteção contra artrópodes. O uso de repelentes compreende a difusão no ar através de spray,

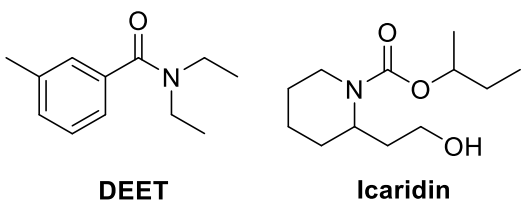
a impregnação em tecidos usados em roupas e redes mosquiteiros e a aplicação direta na pele. O uso tópico é uma das medidas de proteção individual mais eficaz, pois permite a liberação do repelente diretamente no nível em que a picada ocorre, na pele. Os repelentes estão disponíveis em uma variedade de formas, incluindo sprays, loções, cremes e filtros solares (MOORE, *et al*., 2012; KATZ *et al*., 2008).

Há vários produtos à base de repelentes disponíveis no mercado, contendo substâncias ativas sintéticas ou provenientes de fontes naturais. Os repelentes sintéticos tendem a ser mais eficazes, enquanto os de fontes naturais requerem reaplicação frequente devido à alta volatilidade (NERIO *et al.*, 2010).

Apesar de haver muitos produtos disponíveis, existem poucos ingredientes ativos sendo o DEET (N, N-dimetil-3-metilbenzamida) o mais utilizado entre eles (Fig. 1). O DEET possui um amplo espectro de atividade, protegendo contra os mosquitos *Aedes*, *Anopheles* e *Culex*. Embora eficaz, o DEET pode exercer reações tóxicas em algumas circunstâncias e faixas etárias. Além disso, pode danificar plásticos, tecidos sintéticos e superfícies pintadas. (FATHY KATER *et al.*, 2019).

O baixo número de ingredientes ativos existentes e as desvantagens apresentadas pelo DEET justificam a busca de novos repelentes que demonstrem boa eficácia contra vetores de mosquitos, sem efeitos tóxicos ou danos ao meio ambiente (KATZ *et al*., 2008; MOORE *et al*., 2012).

Uma alternativa sintética recente, o repelente comercial Icaridin (fig. 1), tem demostrado baixo número de adversidades e sido tão eficaz quanto o DEET em relação a repelência, mas ainda tem causado alguns efeitos adversos consideráveis, como vômitos e irritações oculares (HASLER *et al.*, 2019), e não é uma alternativa tão barata em países emergentes com baixa industrialização.



DEET **Icaridin**

Figura 1. Estrutura dos repelentes comerciais mais usados

Os derivados de produtos naturais

Derivados da mentona

Em um estudo recente de Iovinella *et al*. (2014), a eficácia do derivado sintético do produto natural mentona foi demonstrada como repelente contra mosquitos *Aedes albopictus*. Nesse trabalho, três derivados sintéticos da mentona (acetais cíclicos) foram planejados para minimizar a volatilidade desse composto, pois essa é a principal limitação ao uso de produtos naturais como repelentes. O foco do estudo foi identificar novas substâncias repelentes que fossem eficientes e tivessem proteção a longo prazo em baixas concentrações. Os resultados obtidos foram muito promissores, uma vez que todos os derivados exibiram atividade e o derivado III (figura 2) mostrou eficácia semelhante ao DEET, mas com duração mais longa. As soluções de 1% de acetal III (142 nmol/cm^2 de pele) e DEET (209 nmol/cm^2 de pele) foram 90% efetivas por 120 e 15 minutos, respectivamente, sugerindo maior tempo de proteção pelo acetal.

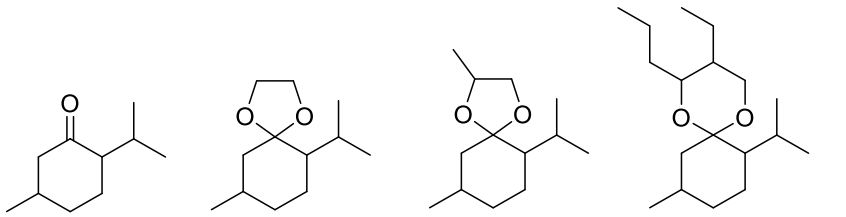


Figura 2. Derivados a partir da mentona, no trabalho de Iovinella (2014).

No entanto, esse trabalho é preliminar, uma vez que apenas três derivados com baixa variabilidade estrutural e propriedades físico-químicas semelhantes foram avaliados. Outra limitação é que os autores usaram a mistura de estereoisômeros da mentona como material de partida e, portanto, obtiveram misturas com pelo menos quatro estereoisômeros para cada acetal. No caso do derivado III, o diol usado possui dois estereocentros adicionais e pode gerar até 16 estereoisômeros possíveis. Além da obtenção de misturas, faltam informações importantes como avaliação da toxicidade e permeabilidade da pele, fundamentais no desenvolvimento de produtos para uso tópico.

A obtenção de mistura e o número de isômeros pode ser um fator problemático em considerações a repelência, pois nem todos os isômeros são ativos, e uma separação destes compostos pode ser difícil (KLUN *et al.,* 2001). Ainda que, Icaridin, seja um repelente comercializado como um racemato, um isômero com a configuração *RS*, mostrou-se como sendo o mais ativo em um estudo de sobreposição molecular e bioensaios. (NATARAJAN *et al.,* 2005). Frente a esses dados, o uso de síntese assimétrica em novos repelentes pode prevenir a formação de misturas e selecionar isômeros ativos, sendo uma estratégia moderna ainda não explorada neste campo. Além disso, o DEET, o composto de referência, é uma molécula com uma disposição espacial específica que parece ser responsável por sua repelência, indicando que o uso de uma estratégia seletiva, pode ser eficaz. (NATARAJAN *et al.,* 2005).

Outros Derivados de compostos naturais

Além da mentona, sabe-se que outros metabólitos secundários, como o limoneno, o eugenol e a cânfora, que ocorrem naturalmente têm propriedades repelentes contra várias espécies de mosquitos (HWANG *et al*., 1985; NERIO *et al*., 2010; POHRK *et al*., 2012).

Uma das moléculas naturais mais promissoras é o limoneno, demonstrado por um estudo QSAR com 4885 descritores, prevendo ter atividade inseticida significativa (SAAVEDRA *et al.*, 2018). Está presente no óleo de citronela, um conhecido repelente de origem natural (MAIA e MOORE, 2011).

O eugenol já é um conhecido repelente sendo utilizado recentemente em estudos do modo de ação olfatório em mosquitos (AFIFY *et al*., 2019). A cânfora é também um conhecido repelente, sendo o maior componente presente no óleo essencial de *Rosmarinus officinalis* (AZEEM *et al*., 2019). Esses compostos também são passíveis de modificações sintéticas visando aumentar a sua repelência intrínseca. Entretanto nenhum estudo foi conduzido utilizando estes compostos de fonte natural e síntese orgânica, visando repelência.

Recentemente, compostos derivados do ácido antranílico têm chamado a atenção por suas propriedades repelentes para artrópodes. Inicialmente elaborada em composições como compostos repelentes de pássaros (ASKHAM, 1992), a atividade dessa classe foi descoberta como provável candidato a repelentes por meio de triagem virtual (KAIN *et al*., 2013), com atividades promissoras que superam o DEET.

Desde esta publicação, uma patente foi depositada, utilizando misturas repelentes contendo pelo menos um composto antranílico (IZADI e FOCK, 2016) Trabalhos recentes (ISLAM *et al*., 2017) demonstram a importância e a potencialidade de derivados antranílicos, como o antranilato de etila, com resultados promissores de repulsão do vetor do vírus Zika, o mosquito *Aedes Aegypti*. Assim, uma formulação contendo um desses compostos antranílico em conjunto com novos candidatos a repelência seria extremamente valiosa, aproveitando seus efeitos em formulações farmacêuticas. Recentemente, o antranilato de etila também foi explorado contra o *Aedes albopictus* para repelência e toxicidade (ZHAO *et al*., 2019). Além disso, o mesmo pode ser encontrado comercialmente, com baixo custo.

Essa natureza do sinergismo é amplamente explorada pelos repelentes, principalmente em compostos naturais. Alguns estudos demonstram as vantagens do sinergismo. Quando misturados óleos essenciais de três diferentes espécies de plantas, ocorreu um aumento de repelência e da irritação em mosquitos, em comparação com as suas contrapartes isoladas (NOOSIDUM *et al.,* 2014; DAS *et al.,* 2015; LEE*,* 2018). Por se tratar de uma prevenção e não de um alvo específico, como enzimas, as formulações podem conter várias moléculas repelentes altamente ativas. O sinergismo ocorre quando a soma dos efeitos das moléculas combinadas é maior que seus efeitos individuais (JIA JIA *et al.*, 2009). Além disso, ao lidar com repelentes, outras características favoráveis podem ser avaliadas, como diminuição da volatilidade na combinação de moléculas. além de outros fatores, como até o odor. Esse tipo de estratégia sinérgica é amplamente utilizada em patentes, onde tira proveito da natureza repelente de um composto natural ou óleo natural em combinação com uma molécula sintética (FRIDRIKS, 2004).

Relação estrutura química e atividade repelente

O estudo de relacionar estruturas químicas e atividade com repelentes é bastante esparso desde o início do novo milênio, sendo boa parte da informação encontrada em uma revisão sistemática realizada em 2010, que agrupa estudos conduzidos nas décadas de 40 e 50, junto com informações importantes sobre as classes químicas encontradas em repelentes. (POHLUCH e COATS, 2010). Em sua maioria, os repelentes encontram-se distribuídos em algumas classes químicas características, com poucas exceções. Essas classes químicas incluem as amidas, os

dióis, piperidinas, ftalatos e compostos vindo de fontes botânicas, como por exemplo, terpenos de baixo peso molecular. As exceções, incluem compostos como benzofuranos e monoálcoois. (POHLUCH e COATS, 2010).

O conhecimento das classes químicas em conjunto com o surgimento de novas tecnologias, como *docking molecular* e principalmente QSAR, tem favorecido o desenvolvimento de novos compostos promissores. O uso de QSAR tem sido cada vez mais frequente, sendo empregados o uso de vários descritores matemáticos em conjunto com bibliotecas de compostos com ou sem atividade repelente. Em sua maioria, o DEET é utilizado como base teórica nestes estudos. (WANG *et al.,* 2017; BASAK e BHATTACHARJEE, 2018; DEVILLERS, 2018; DOUCET *et al.,* 2019). Resultados advindos destes estudos QSAR, sugerem estruturas promissoras de amidas, carboxamidas, piperidinas e até mesmo terpenos que podem ser utilizados como *scaffolds* para síntese e melhoramento das atividades previstas. Um exemplo destes resultados QSAR é o limoneno (SAAVEDRA *et al.,* 2018)

Com estes estudos preditivos, a descoberta de novos candidatos, e até mesmo de novas classes *repelentes*, pode ser realizada de forma mais racional e eficaz.

Repelência e o sistema olfatório dos mosquitos

Uma estratégia moderna para predição e validação de atividades repelentes consiste no uso de *docking* molecular para proteínas de ligação de odorantes no sistema olfatório de mosquitos. As proteínas de ligação de odorantes (*Odorant Binding Protein,* OBP) estão presentes na hemolinfa e são consideradas os “portões” no sistema olfativo, onde reconhecem e encapsulam os odorantes hidrofóbicos, que são encaminhados para os receptores de odor. (OLIFERENKO *et al*., 2013). Os receptores de odor provocam uma ampla gama de atividades bioquímicas e uma complexa interação na rede neural com outros receptores, criando uma resposta "espacial" específica que caracteriza o odor. Essa resposta depende da concentração, classe química dos odorantes entre outros parâmetros (HALLEM e CARLSON, 2006). A OBP1 é escolhida como principal alvo para estudos computacionais, é super-expressa em mosquitos fêmeas, é o primeiro passo no reconhecimento olfativo nos mosquitos *Anopheles* e *Aedes* (BIESSMANN *et al.*, 2010; MAO *et al.*, 2010).

A OBP1, conhecida como AgamOBP1 (de *Anopheles gambiae*), é a mais investigada nos métodos *in silico* para prever possíveis moléculas repelentes ativas e

para criar uma correlação entre a repelência e sua interação com a OBP1. Alguns trabalhos nos últimos anos exploraram essa abordagem, creditada às novas estruturas cristalográficas do AgamOBP1 complexadas com repelentes, como DEET e Icaridin. Esses estudos trouxeram conhecimento nas regiões do AgamOBP1 onde o DEET apresenta interações, juntamente com uma modelagem baseada em estruturas químicas promissoras (*leads*) (TSITSANOU *et al*., 2012). Novos modos de ligação alternativos no OBP1 foram revelados através do complexo cristalográfico da OBP1 e Icaridin (DRAKOU *et al*., 2017) e um modelo refinado com triagem e *docking* combinados, baseados em ligantes do AgampOBP1 com base nas interações do DEET e Icaridin foi proposto, com informações particulares do modo de ligação e repelência (THIREOU *et al*., 2018). A atualização dessas informações, bem como a atualização destes modelos, pode gerar ferramentas poderosas e com alto nível de eficiência para predição de novas moléculas repelentes.

Embora esses estudos apresentem informações valiosas para uma abordagem racional para novos candidatos a repelentes, essas informações estão focadas no *Anopheles gambiae* OBP1, e existem poucos trabalhos que se concentram no *Aaedes aegypti* com estudos *in silico*. Os trabalhos mais recentes têm como base a AaegOBP1 (Figura 3) o equivalente ao AgamOBP1 no *Aaedes aegypti* (OLIFERENKO *et al.*, 2013; BORGES *et al.*, 2019, PORTILLA-PULIDO *et al.,* 2019), com bons resultados experimentais que correlacionam com as predições realizadas, ainda que não mais eficazes que os repelentes comerciais. Estes trabalhos, apresentam informações valiosas no modo de ligação de moléculas ativas e nas regiões e bolsões da proteína, que ajudarão a entender como a repelência funciona nesse estágio, podendo também fornecer dados para refinar e desenvolver repelentes mais potentes no futuro. Além disso, o uso da estratégia *in silico* em conjunto com estudos *in vitro* serão úteis para validar os resultados e aumentar a racionalidade do trabalho.

Justificativa do tema

Apesar das diversas vantagens dos repelentes como produtos fáceis de se encontrar e de fácil aplicação, o alto custo perante a população brasileira, bem como a falta de instruções específicas tende a diminuir o uso de repelentes perante certos grupos sociais (MELO, 2019).

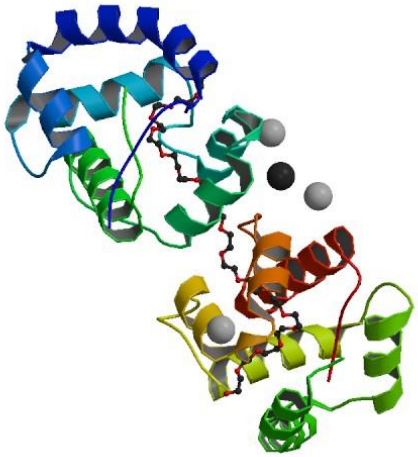


Figura 3. Estrutura cristalizada da proteína AaegOBP1. Fonte: PDB (Protein Data Bank), código 3K1E.

Esse projeto irá utilizar compostos de partida de fácil acesso. Foram planejadas cinco séries de derivados a partir de: cânfora, mentona, limoneno, eugenol e ésteres antranilados.

Até o momento, nenhuma das substâncias propostas neste projeto, apesar da síntese já ter sido reproduzida para alguns derivados, foi avaliada quanto à atividade repelente, de acordo com a literatura.

Todos os compostos serão testados na forma de triagem em mosquitos no Laboratório coordenado pelo Prof. Dr. Alexandre de Almeida e Silva na Universidade Federal de Rondônia (UNIR), cujo grupo tem ampla experiência. Além dos compostos sintetizados, serão testados os produtos de partida, e DEET como controle positivo.

Com a disponibilidade do uso de compostos de origem natural e a possibilidade de uma rota sintética seletiva para os produtos esperados, a síntese desses derivados se desdobra como uma alternativa aos repelentes 100% sintéticos, buscando procurar derivados menos agressivos ao ambiente e mais ativos em relação aos repelentes comerciais sintéticos existentes, justificando sua síntese.

Outro fator importante é que a prevenção é necessária em um país tropical como o Brasil e de proporções continentais. O uso de repelentes de baixo custo com materiais de fácil acesso permite uma melhor distribuição e acesso desses possíveis repelentes à população em geral, a fim de combater epidemias como as ocorridas em

2015 pelo vírus Zika. Além disso, na continuação desse projeto, os compostos sintetizados serão avaliados como larvicidas e inseticidas, e também serão realizados estudos de *docking molecular* na AaegOBP1, visando ampliar a gama de potenciais aplicações desses compostos.

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CAPÍTULO 1. Synthesis and structure–activity relationships of natural based promising repellents for *Aedes aegypti*

This manuscript will be submitted to *Pest Management Science*.

1 INTRODUCTION

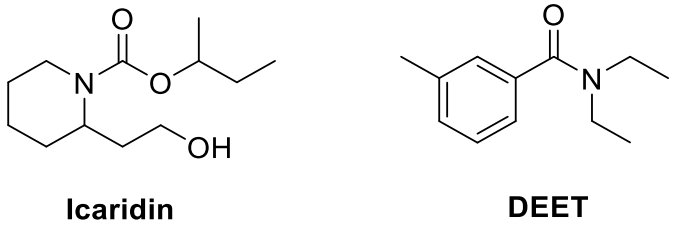
In the last years, the emergence of arboviral epidemic has caused great concern in respect of public health across many countries and flagged a red alert. The arboviruses could be transmitted to vertebrate hosts by hematophagous arthropods vectors. Among them, mosquitoes belonging to the genre *Anopheles* and *Aedes* are accountable for transmitting serious diseases such as malaria, dengue and zika (FAUCI and MORENS, 2016; PETERSEN *et al*., 2016; WILDER-SMITH *et al*, 2017)

While combating the vector is still inefficient in many countries, and due to the lack of available Zika virus vaccines, it is recommended to use preventive measures such as avoiding mosquito exposure during the day and personal protection with the use of repellents. These measures are especially important for pregnant women, based on the association between virus infection and newborn microcephaly cases (BOGOCH *et al*., 2016).

Topical repellents are considered the first line of defense against transmitting insects being cheap and having an easy application for protection against arthropods. The use of repellents comprises their diffusion into the air through spray, their impregnation in fabrics used in clothing and mosquito nets and their direct application to the skin. Repellents are available as sprays, lotions, creams, and sunscreens (KATZ *et al*., 2008; MOORE, *et al*., 2012).

Synthetic repellents have the tendency to be more effective, while those of natural sources, often require frequent application due to high volatility. Despite the many commercial products available, there are few synthetic active ingredients, usually DEET and Icaridin (Fig. 1). DEET (N, N-dimethyl-3-methylbenzamide) is the most used, having a broad spectrum of activity, protecting against *Aedes, Anopheles* and *Culex* mosquitoes. Although effective, DEET can exert toxic reactions in some circumstances and age groups. (KATZ *et al*., 2008; MOORE *et al.,* 2012). Along with DEET, Icaridin is another popular repellent. Commercialized as a racemate, is a fully synthetic repellent with small side effects, being a good candidate to replace DEET completely (FATHY *et al*., 2019).

The low number of existing active ingredients and the disadvantages presented by DEET justify the search for new repellents showing good efficacy against mosquito vectors with less toxic effects.



Icaridin **DEET**

Fig. 1. Structure of the most used commercial repellents

In a recent study by Iovinella *et al.* (2014), the efficacy of synthetic derivative of the natural product menthone was demonstrated as a repellent against *Aedes albopictus* mosquitoes. In this work, menthone cyclic acetals were designed to minimize the volatility of menthone, as this, is the main limitation on the use of natural products as repellents. One of these derivatives showed similar efficacy to DEET with longer duration effect.

In addition to menthone, other naturally occurring metabolites as limonene and eugenol are known to have repellent properties against different mosquito species. (NERIO *et al.,* 2010; POHRK *et al.*, 2012; SAAVEDRA *et al*., 2018 ; AFIFY *et al.*, 2019).

Compounds derived from anthranilic acid have also drawn attention for their repellent properties for arthropods. Initially elaborated into compositions as bird repellent compounds (ASKHAM, 1992), then this chemical class was discovered as potential repellents through virtual screening (KAIN et al. 2013). Recently, ethyl anthranilate was also explored against *Aedes albopictus* for repellency and toxicity (ZHAO, 2019).

Therefore, the main objective of this study is to make structural changes in natural products that have limited repellent activity aiming at the discovery of new more powerful and low cost repellents. As a secondary objective, it is also intended to modify the ethyl anthranilate structure that showed a repellent and relevant activity to better determine the potential of this class.

2 MATERIALS AND METHODS

2.1 Chemistry

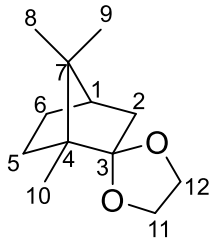
D-Camphor, D-limonene, L-menthone and 2-nitrobenzaldehyde were purchased from Sigma-Aldrich. Eugenol was bought from commercial sources and purified by vacuum distillation (160-165 ºC/ 4 mmHg) before use. All other reagents were obtained from commercial suppliers. Solvents used in column chromatography were bought from commercial sources and distillated before use. Anhydrous dichloromethane and toluene were obtained by treatment with molecular sieves 4 Å (pellet, 3.2 mm). All reactions were monitored by thin layer chromatography (TLC) using silica gel on TLC aluminum foil, 60 Å with fluorescent indicator 254 nm. Column chromatography was carried out using Fluka silica gel 0.063-0.2mm. All ^{1}H and ^{13}C NMR data were recorded by Bruker Avance III 400 at 298K. Chemical shifts (δ) were reported in parts per million (ppm) relative to the internal standard tetramethylsilane (TMS). High Resolution Mass Spectra (HRMS) data were recorded by Bruker Impact II Ultra High-Performance Liquid Chromatography (UHPLC) coupled with mass spectrometer equipped with electron spray ionization (ESI) and Quadrupole Time of Flight (QTOF). Gas chromatograph coupled to impact mass spectrometer (GC-MS) was used.as ionization mode and quadrupole analyzer, model Shimadzu GCMS-QP2010 SE, equipped with Restek RTX-5MS column (30 m x 0.25 mm, 0.25 µm). Synthetic procedure and characterization are described below. All NMR spectra are provided in Supplementary Material.

2.2 General procedure for obtention of D-camphor acetal (1a and 1b) and L-menthone acetal (3a and 3b):

In a 50 mL reaction flask with aid of Dean Stark apparatus for continuous removal of water, a mixture of 1 g of L-mentone or D-camphor (1 eq.), anhydrous toluene (20 mL) and 3 eq. of suitable diol were added together with paratoluenesulfonic acid (PTSA) in catalytic amounts (240 mg, 10 mol %). The reaction mixture was maintained under reflux for 48 h and allowed to reach room temperature after completion time. The mixture was washed with saturated aqueous $NaHCO_3$ solution (20 mL x 2), water (20 mL x 1) and brine (20 mL x 1). The organic layer was dried over anhydrous Na_2SO_4

then concentrated by rotary evaporation. The residue was purified by column chromatography with cyclohexane/ethyl acetate (95:5, v/v) as eluent to obtain the desired products. Conditions adapted from Caron (2011).

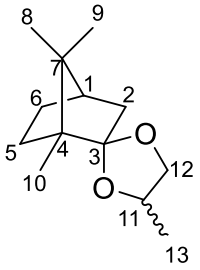
1a – (1S,4R)-1,7,7-trimethylspiro[bicyclo[2.2.1]heptane-2,2'-[1,3]dioxolane] (camphor acetal) :

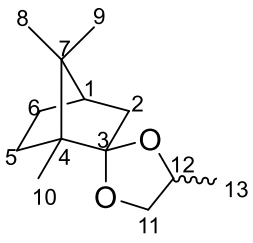


1a

Colorless liquid. Yield = 33%. It was obtained using camphor and ethylene glycol as the diol. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ (ppm) = 3.91 – 3.66 (m, 4H*,* H-11, H-12), 1.98 (dt, *J* = 13.2, 3.9 Hz, 1H, H-2), 1.86 (ddd, *J* = 12.9, 9.5, 3.7 Hz, 1H, H-5), 1.70 – 1.60 (m, 2H, H-1, H-6), 1.40 – 1.26 (m, 2H, H-2', H-5'), 1.21 – 1.10 (m, 1H, H-6'), 0.97 (s, 3H), 0.80 (s, 3H), 0.75 (s, 3H). **^{13}C NMR APT** (101 MHz, CDCl3) δ (ppm) = 117.1 (C-3, C qt. APT), 64.8 (C-11, CH_2), 63.6 (C-12, CH_2), 52.1 (C-4, C qt.), 48.0 (C-7, C qt.), 44.9 (C-1, C qt.), 44.6 (C-2, CH_2), 29.4 (C-5, CH_2), 26.9 (C-6, CH_3), 20.4 (C-8, CH_3), 20.3 (C-9, CH3), 9.7 (C-10, CH_3). HRMS-ESI (*m/z*) $[M+ H]^+$ Calcld. for $C_{12}H_{20}O_2$, 197.1536; found 197.1531;

1b - (1S,2S,4R) -1,4',7,7-tetramethylspiro[bicyclo[2.2.1]heptane-2,2'-[1,3]-dioxolane] (camphor acetal) :

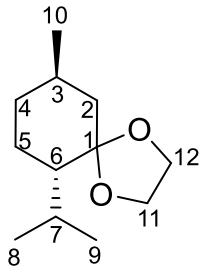




1b **1c**

Colorless liquid. It was obtained using camphor and propylene glycol as the diol. Mixture with **1c** and pair of diasterisomers for each. Yield = 30%. Mixture confirmed in HRMS (no ratio).

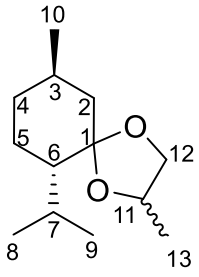
3a- (6S,9R)-6-isopropyl-9-methyl-1,4-dioxaspiro[4.5]decane :

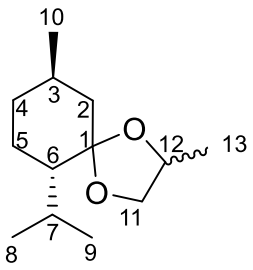


3a

Colorless liquid. It was obtained using L-menthone and ethylene glycol as the diol. Mixture with isomenthone. Yield: 87%. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ (ppm): 3.96 – 3.77 (m, 4H, H-11, H-12), 2.02 (pd, *J* = 6.9, 1.6 Hz, 1H, H-5), 1.72 – 1.62 (m, 2H, H-2, H-4), 1.56 (m, 5H, 2H, H-7, H-5'), 1.38 – 1.24 (m, 2H, H-6, H-3), 1.00 – 0.85 (m, 2H, H-2', H-4'), 0.83 (d, *J* = 7.0 Hz, 3H, H-10), 0.81 (d, *J* = 6.5 Hz, 3H, H-8), 0.79 (d, *J* = 7.0 Hz, 3H, H-9). **^{13}C NMR APT** (101 MHz, CDCl3) δ (ppm) = 111.8 (C-1), 64.6 (C-11), 64.3 (C-12), 49.2 (C-6), 44.7 (C-2), 34.5 (C-4), 30.6 (C-7), 24.6 (C-3) , 23.4 (C-5), 23.4 (C-10), 22.1 (C-8), 18.3 (C-9). The mixture was confirmed by GC with approximately 95:5 ratio.

3b- (6S, 9R)-6-isopropyl-2,9-dimethyl-1,4-dioxaspiro[4.5]decane :





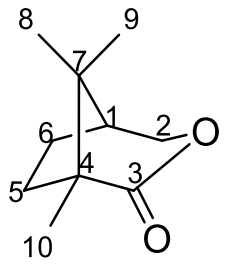
3b **3c**

Colorless liquid. It was obtained using L-menthone and propylene glycol as the diol. Mixture with 3c and pair of diasterisomers for each Yield: 72%.The mixture was confirmed by GC with approximately 4:3:2:1 ratio.

2.3. Procedure for obtain D-camphor lactone (2) by Baeyer-Villiger Oxidation:

In a 25 mL reaction flask, a mixture of 0.3366 g (1 eq.) of D-Camphor and meta-chloroperoxybenzoic acid (m-CPBA) 0.89 g (2.5 eq.) were added in 10 mL of anhydrous dichloromethane with triflic acid (tf-OH) 0.18 mL (0.3 eq.) as catalyst. The reaction mixture was maintained under agitation for 3 h at room temperature. Upon completion time, the mixture was quenched with a 2 N aqueous NaOH solution. Extraction was made using dichloromethane (2 x 15 mL). The organic layer was dried over anhydrous Na_2SO_4, then concentrated by rotary evaporation. The residue was purified by column chromatography with cyclohexane / ethyl acetate (8:2, v/v) as eluent to obtain the desired product. Conditions adapted from Forest (2003).

2- (1S,5S)-1,8,8-trimethyl-3-oxabicyclo[3.2.1]octan-2-one :



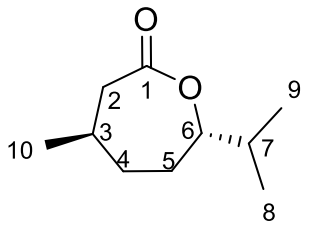
2

White solid. Yield: 61%. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ (ppm) = 4.53 – 4.42 (m, 1H, H-2^{I}), 4.10 (d, J = 10.8 Hz, 1H, H-2^{II}), 2.21 – 1.72 (m, 5H, H1, H6, H5), 1.16 (s, 3H, H-10), 1.10 (s, 3H, H-9), 0.98 (s, 3H, H-8). **^{13}C NMR** (101 MHz, $CDCl_3$) δ (ppm) = 177.01 (C-3), 73.97 (C-2), 53.52 (C-4), 44.36 (C-1), 42.06 (C-7), 36.01 (C-5), 26.82 (C-6), 22.24 (C-9), 19.75 (C-8), 14.18 (C-10). HRMS-ESI (*m/z*) $[M+ H]^+$, calcld. for $C_{10}H_{16}O_2$, 168.1150; found 168.1150.

2.4. Procedure for obtain L-menthone lactone (4) by Baeyer-Villiger Oxidation:

In 50 mL reaction flask, a mixture of 0.2 g (1 eq) of L-menthone and 0.6 g (3 eq.) of meta-chloroperoxybenzoic acid (m-CPBA) were added together in 25 mL of anhydrous dichloromethane with 0.02 g (0.1 eq.; 10%) of PTSA as catalyst. The reaction mixture was maintained under reflux for 24 h. After completion time, it was allowed to reach room temperature with addition of 15 mL of dichloromethane. Organic phase was extracted with 20 mL of saturated aqueous $NaHSO_3$ solution (1x), 20 mL of saturated aqueous $NaHCO_3$ solution (1x) and 10 mL of distilled water. The organic layer was dried over anhydrous Na_2SO_4, then concentrated by rotary evaporation. The residue was purified by column chromatography with cyclohexane/ethyl acetate (9:1, v/v) as eluent to obtain the desired product. Conditions adapted from Cristau (2005).

4- (4R,7S)-7-isopropyl-4-methyloxepan-2-one :



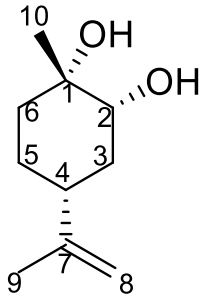
4

White Solid. Yield : 41%. **^{1}H NMR** (400 MHz, Chloroform-d) δ 4.07 (dd, J = 9.3, 4.4 Hz, 1H, H-6), 2.57 (dd, J = 13.1, 11.8 Hz, 1H, H-2'), 2.46 (dq, J = 13.4, 1.6 Hz, 1H, H-2), 2.00 – 1.87 (m, 2H, H-4), 1.89 – 1.82 (m, 2H, H-5), 1.66 – 1.53 (m, 1H, H-3), 1.39 – 1.24 (m, 1H, H-7), 1.05 (d, J = 6.7 Hz, 3H, H-10), 0.98 (t, J = 6.3 Hz, 6H, H-9 and H-8). **^{13}C NMR** (101 MHz, $CDCl_3$) δ (ppm) = 174.9 (C-1), 84.6 (C-6), 42.5 (C-2), 37.3 (C-4), 33.2 (C-5), 30.9 (C-3), 30.4 (C-7), 23.9 (C-10), 18.3 (C-9), 17.0 (C-8). HRMS-ESI (*m/z*) $[M+ H]^+$, calcld. for $C_{10}H_{18}O_2$, 170.1307; found 170.1307;

2.5. General procedure for obtain the diols of limonene (5a-b) and eugenol (8a-b) by Sharpless Asymmetric Dihydroxylation:

In a 50 mL reaction flask, the desired compound (limonene or eugenol) (1 eq. 0.0073 mol) was added together with 20 ml of t-BuOH and 20 ml of water. The mixture was cooled to 0º in an ice bath. Upon cooling, 7.24 g of K_2CO_3 (3 eq. 0.022mol) and 3.04 g of K_3Fe [CN_6] (3 eq. 0.022 mol) were added in the reaction mixture. Then, (DHQD)2-PHAL (40 mg, 4% eq.) and $K_2OsO_2(OH)_4$ (40 mg, 4% eq.) were added in the heterogeneous mixture. The reaction was maintained under vigorous stirring at 0ºC for 16 h. After the completion, the reaction mixture was diluted with 20 mL of 20% aqueous NaCl solution and extracted with 20 mL of ethyl acetate. After separation, the aqueous phase was extracted with 20 mL of ethyl acetate. The organic phases were combined and dried with Na_2SO_4 and then concentrated by rotary evaporation. The residue was purified by column chromatography with cyclohexane/ ethyl acetate (1:1, v/v) as eluent to obtain the desired products. Conditions adapted from Liao (1999).

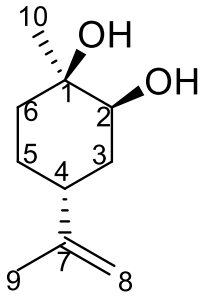
5a- (1S,2R,4R)-1-methyl-4-(prop-1-en-2-yl)cyclohexane-1,2-diol :



5a

White solid. Yield = 41%. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 4.73 – 4.60 (m, 2H, H-8), 3.38 (dd, *J* = 11.5, 4.6 Hz, 1H, H-2), 2.90 (s, 1H, OH), 2.54 (s, 1H, OH), 1.92 (dp, *J* = 11.6, 3.9 Hz, 1H, H-4), 1.85 – 1.73 (m, 2H, H-6, H-3), 1.72 (s, 3H, H-9), 1.50 (qdd, *J* = 12.1, 8.2, 5.2 Hz, 3H, H-3', H-5, H-5'), 1.41 – 1.30 (m, 1H, H-6'), 1.25 (d, *J* = 1.3 Hz, 3H, H-10).**^{13}C NMR APT** (101 MHz, $CDCl_3$) δ 149.05 (C-7, C qt.), 108.89 (C-8, CH_2), 75.16 (C-2, CH), 70.86 (C-1, C qt.), 43.71 (C-4, CH), 37.31 (C-6, CH_2), 35.56 (C-3, CH_2), 27.16 (C-10, CH_3), 26.17 (C-5, CH_3), 20.83 (C-9, CH_3).

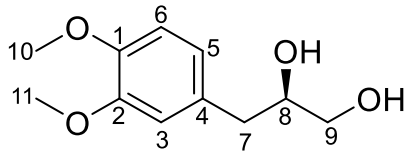
5b- (1R,2S,4R)-1-methyl-4-(prop-1-en-2-yl)cyclohexane-1,2-diol :



5b

Dark grey oil. Mixture of **5b** with **5a**. Yield = 31%. The mixture was confirmed by GC, with approximately 1:1 ratio of **5b** and **5a**.

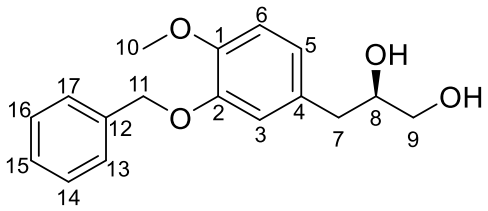
8a- (R)-3-(3,4-dimethoxyphenyl)propane-1,2-diol :



8a

White solid. Yield = 32%. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ (ppm): 6.71 (d, *J* = 8.1 Hz, 1H, H-5), 6.69 – 6.64 (m, 2H, H-6, H-3), 3.78 (s, 3H, H-10), 3.85 (dd, *J* = 7.2, 3.0 Hz, 1H, H-8), 3.76 (s, 3H, H-11), 3.54 (dd, *J* = 11.5, 2.9 Hz, 1H, H-9'), 3.37 (dd, *J* = 11.4, 7.1 Hz, 1H, H-9), 2.61 (s, 1H, H-7'), 2.59 (s, 1H, H-7). **^{13}C NMR APT** (101 MHz, CDCl3) δ (ppm): 148.8 (C-2, C qt.), 147.6 (C-1, C qt.), 130.5 (C-4, C qt.), 121.3 (C-5, CH), 112.6 (C-6, CH), 111.3 (C-3, CH), 73.2 (C-8, CH), 65.8 (C-9, CH_2), 55.9 (C-10, CH_3), 55.8 (C-11, CH_3), 39.2 (C-7, CH_2). HRMS-ESI (*m/z*) $[M+H]^+$, calcld. for $C_{11}H_{16}O_4$, 210.1049; found 210.1043.

8b- (R)-3-(3-(benzyloxy)-4-methoxyphenyl)propane-1,2-diol :



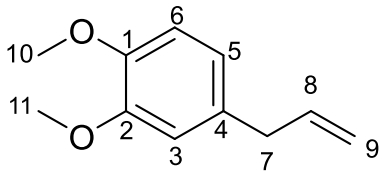
8b

Yellow solid. Yield = 55%. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 7.45 (d, *J* = 7.3 Hz, 2H, H-17, H-13), 7.37 (t, *J* = 7.4 Hz, 2H, H-16, H-14), 7.31 (t, *J* = 7.2 Hz, 1H, H-15), 6.85 – 6.75 (m, 2H, H-6, H-5), 6.68 (dd, *J* = 8.1, 1.5 Hz, 1H, H-3), 5.11 (s, 2H, H-11), 3.86 (s, 4H, H-10, H-8), 3.60 (d, *J* = 10.6 Hz, 1H, H-9'), 3.49 – 3.39 (m, 2H, H-9, OH), 2.66 (d, *J* = 7.1 Hz, 2H, H-7). **^{13}C NMR APT** (101 MHz, $CDCl_3$) δ 149.62 (C-2, C qt.), 146.86 (C-1, C qt.), 137.23 (C-12, C qt.), 131.15 (C-4, C qt.), 128.57 (C-16, C-14, CH), 127.88 (C-15, CH), 127.35 (C-17, C-13, CH) 121.32 (C-5, CH), 114.22 (C-6, CH), 113.22 (C-3, CH), 73.19 (C-8, CH), 71.14 (C-11, CH_2), 65.90 (C-9, CH_2), 56.04 (C-10, CH_3), 39.30 (C-7, CH_2). HRMS-ESI (*m/z*) $[M+ H]^+$, calcld. for $C_{17}H_{20}O_4$, 288.1362; found 288.1356.

2.6. General procedure for eugenol ethers (7a-b) by Williamson Ether synthesis:

In 100 ml Schlenk flask, 2 g of eugenol (1 eq., 0.0122 mol) was added together with 5 mL of anhydrous DMF (dimethylformamide) and 3.37 g of K_2CO_3 (2 eq. 0.0244 mol). The reaction mixture was kept under stirring for one hour for activation of eugenol at room temperature. After one hour, 2.28 mL of methyl iodide (3 eq., 0.0365 mol) were added and the reaction was maintained sealed under vigorous stirring in reflux, overnight. After reaction time, the mixture was diluted with 100 mL of ethyl acetate. The organic phase was washed with a 100 mL of 0.5 M aqueous NaOH solution (1x), water (1x), then dried with Na_2SO_4 and concentrated by rotary evaporation. No further purification was necessary. Conditions adapted from Sharp (2006).

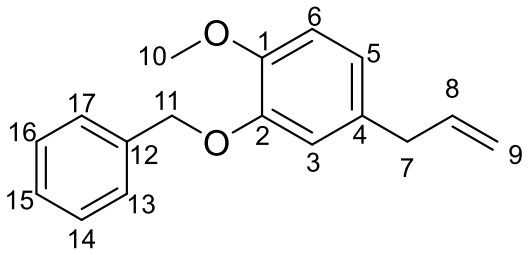
7a- 4-allyl-1,2-dimethoxybenzene :



7a

Yellow liquid. Yield: 99%. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ (ppm): 6.77 (d, *J* = 8.6 Hz, 1H, H-5), 6.71 (dd, *J* = 4.2, 2.4 Hz, 2H, H-6, H-3), 6.02 – 5.90 (m, 1H, H-8), 5.12 – 5.04 (m, 2H, H-9, H-9'), 3.83 (s, 3H, H-10), 3.81 (s, 3H, H-11), 3.33 (s, 1H, H-7), 3.32 (s, 1H, H-7'). **^{13}C NMR APT** (101 MHz, $CDCl_3$) δ (ppm): 148.9 (C-2, C qt.), 147.4 (C-1, C qt.), 137.6 (C-8, CH), 132.5 (C-4, C qt.), 120.4 (C-5, CH), 115.4 (C-9, CH_2), 111.9 (C-6, CH), 111.3 (C-3, CH), 55.7 (C-10, CH_3), 55.6 (C-11, CH_3), 39.7 (C-7, CH_2). HRMS-ESI (*m/z*) [M+ H]$^+$, calcld. for $C_{11}H_{14}O_2$, 178.0994; found 178.0988.

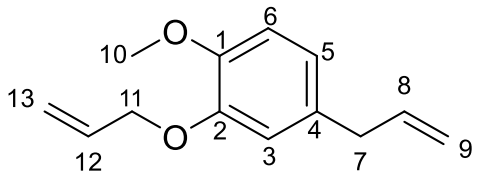
7b – 4-allyl-2-(benzyloxy)-1-methoxybenzene :



7b

Yellow Liquid. Yield = 99%. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ (ppm) = 7.44 – 7.37 (m, 2H, H-17, H-13), 7.35 – 7.28 (m, 2H, H-16, H-14), 7.28 – 7.22 (m, 1H, H-15), 6.78 (d, *J* = 8.1 Hz, 1H, H-5), 6.71 (d, *J* = 2.0 Hz, 1H, H-6), 6.63 (dd, *J* = 8.1, 2.0 Hz, 1H, H-3), 5.93 (ddt, *J* = 16.8, 10.0, 6.7 Hz, 1H, H-8), 5.08 (s, 2H, H-11), 5.07 – 5.01 (m, 2H, H-9, H-9'), 3.82 (s, 3H, H-10), 3.29 (d, *J* = 6.7 Hz, 2H, H-7, H-7'). **^{13}C NMR APT** (101 MHz, $CDCl_3$) δ (ppm) = 149.73 (C-2, C qt.), 146.65 (C-1, C qt.), 137.75 (C-8, CH), 137.50 (C-12, C qt.), 133.37 (C-4, C qt.), 128.60 (C-16, C-14, CH), 127.85 (C-15, CH), 127.39 (C-17, C-13, CH), 120.54 (C-5, CH), 115.77 (C-9, CH_2), 114.34 (C-3, CH), 112.52 (C-6, CH), 71.25 (C-11, CH_2), 56.00 (C-10, CH_3), 39.94 (C-7, CH_2). HRMS-ESI (*m/z*) [M+ H]$^+$, calcld. for $C_{17}H_{18}O_2$, 254.1307; found 254.130.

7c- 4-allyl-2-(allyloxy)-1-methoxybenzene :



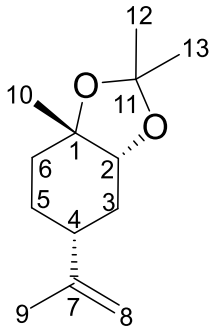
7c

Yellow Liquid. Yield : 99% **.^{1}H NMR** (400 MHz, Chloroform-*d*) δ (ppm): 6.76 (d, J = 8.1 Hz, 1H, H-5), 6.68 (d, J = 2.1 Hz, 1H, H-6), 6.65 (dd, J = 8.1, 2.1 Hz, 1H, H-3), 6.08 – 5.86 (m, 2H, H-12, H-8), 5.35 (dq, J = 17.3, 1.6 Hz, 1H, H-13), 5.21 (dq, J = 10.5, 1.4 Hz, 1H, H-13'), 5.10 – 4.99 (m, 2H, H-9, H-9'), 4.50 (dt, J = 5.4, 1.6 Hz, 2H, H-11, H-11'), 3.78 (s, 3H, H-10), 3.28 (dt, J = 6.8, 1.7 Hz, 2H, H-7, H-7').**^{13}C NMR APT** (101 MHz, $CDCl_3$) δ (ppm): 149.5 (C-2, C qt.), 146.4 (C-1, C qt.), 137.7 (C-12, CH), 133.6 (C-8, CH), 133.0 (C-4, CH), 120.4 (C-5, CH), 117.5 (C-13, CH_2), 115.6 (C-9, CH_2), 113.7 (C-3, CH), 112.3 (C-6, CH), 69.9 (C-11, CH_2), 55.7 (C-10, CH_3), 39.8 (C-7, CH_2). HRMS-ESI (*m/z*) $[M+ H]^+$, calcld. for $C_{13}H_{16}O_2$, 204.1150; found 204.1145.

2.7. General procedure for obtention of limonene (6a-b) and eugenol acetals (9a-b):

In a 50 mL reaction flask, 15 mL of acetone, 5 mL (15 eq.) of dimethoxy propane (DMP) and 0.382g of the suitable diol were added together. After, 50 µL (0.2 eq.) of $BF_3.Et_2O$ was added and the reaction mixture was kept under stirring for 8 h or until a permanent change of color. Upon completion, 1 mL of Et_3N was added to the mixture which was concentrated by rotary evaporation. The residue was directly purified by column chromatography with cyclohexane/ ethyl acetate (95:5, v/v) as eluent to obtain the desired product. Conditions adapted from Andrade (2014).

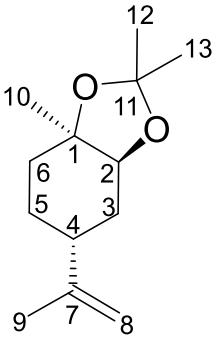
6a-(3aS,6R,7aR)-2,2,3a-trimethyl-6-(prop-1-en-2-yl)-hexahydrobenzo[d][1,3]-dioxole :



6a

Yellow Liquid. Yield = 88% **^{1}H NMR** (400 MHz, Chloroform-*d*) 4.75 (dq, *J* = 1.9, 0.9 Hz, 1H, H-8), 4.71 (p, *J* = 1.6 Hz, 1H, H-8), 3.95 (dd, *J* = 7.2, 6.1 Hz, 1H, H-2), 2.09 – 2.00 (m, 1H, H-4), 1.96 – 1.92 (m, 1H, H-6), 1.92 – 1.87 (m, 1H, H-3), 1.73 (s, 3H, H-9), 1.71 (m, 1H, H-3'), 1.63 – 1.55 (m, 1H, H-5), 1.54 – 1.49 (m, 1H, H-5'), 1.47 (d, *J* = 0.8 Hz, 3H, H-10), 1.45 – 1.42 (m, 1H, H-6'), 1.40 (d, *J* = 0.8 Hz, 3H, H-13), 1.33 (s, 3H, H-12).**^{13}C NMR APT** (101 MHz, $CDCl_3$) δ 149.3 (C-7, C qt.), 109.2 (C-8, CH_2), 107.6 (C-11, C qt.), 79.7(C-2, CH), 79.4 (C-1, C qt.), 39.1 (C-4, CH), 34.2 (C-6, CH_2), 31.9 (C-3, CH_2), 28.9 (C-10, CH_3), 27.8 (C-13, CH_3), 27.4 (C-12, CH_3), 24.9 (C-5, CH_2), 20.8 (C-9, CH_3). HRMS-ESI (*m/z*) $[M+H]^+$, calcld. for $C_{13}H_{22}O_2$, 210.1620; found 210.1614;

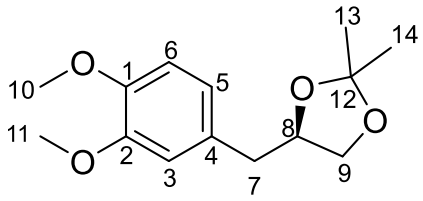
6b-(3aR,6R,7aS)-2,2,3a-trimethyl-6-(prop-1-en-2-yl)hexahydrobenzo[d][1,3]-dioxole :



6b

Yellow liquid. Mixture with **6b** and **6a**. Yield = 23%. The mixture was confirmed by GC, with approximately 1:1 ratio of **6b** and **6a**.

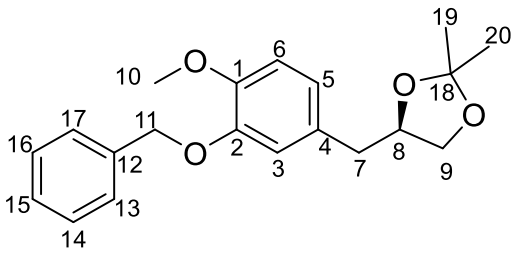
9a- (R)-4-(3,4-dimethoxybenzyl)-2,2-dimethyl-1,3-dioxolane :



9a

Yellow Liquid. Yield = 63%. **^{1}H NMR** (400 MHz, Chloroform-d) δ (ppm): 6.81 – 6.60 (m, 3H, H-5, H-6, H-3), 4.21 (td, J = 6.5, 2.6 Hz, 1H, H-9'), 3.78 (s, 3H, H-10), 3.75 (s, 3H, H-11), 3.54 (td, J = 7.7, 3.3 Hz, 1H, H-9), 2.85 (ddd, J = 13.5, 6.0, 3.1 Hz, 1H, H-7), 2.64 (dq, J = 10.7, 3.8, 3.4 Hz, 1H, H-7'), 1.35 (s, 3H, H-13), 1.27 (s, 3H, H-14). **^{13}C NMR APT** (101 MHz, $CDCl_3$) δ 148.8 (C-1, C qt.), 147.7 (C-2, C qt.), 130.0 (C-4, C qt.), 121.1 (C-5, CH), 112.4 (C-6, CH), 111.2 (C-3, CH), 108.9 (C-12, C qt.), 76.7 (C-8, CH), 68.8 (C-9, CH_2), 55.7 (C-11, CH_3), 55.6 (C-10, CH_3), 39.5 (C-7, CH_2), 27.0 (C-13, CH_3), 25.6 (C-14, CH_3). HRMS-ESI (*m/z*) $[M+ H]^+$, calcld. for $C_{14}H_{20}O_4$, 252.1362; found 252.1356;

9b- (R)-4-(3-(benzyloxy)-4-methoxybenzyl)-2,2-dimethyl-1,3-dioxolane :



9b

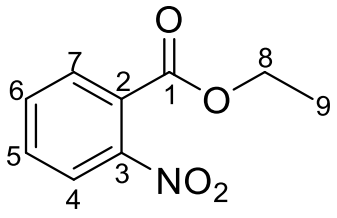
Yellow Liquid. Yield = 55%. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ (ppm) = 7.45 (s, 2H, H-17, H-13), 7.38 (t, *J* = 7.4 Hz, 2H, H-16, H-14), 7.31 (dd, *J* = 14.2, 7.0 Hz, 1H, H-15), 6.86 – 6.77 (m, 2H, H-6, H-5), 6.70 (dd, *J* = 8.1, 1.7 Hz, 1H, H-3), 5.15 (s, 2H, H-11), 4.37 – 4.26 (m, 1H, H-8), 3.98 (dd, *J* = 8.1, 5.9 Hz, 1H, H-9'), 3.91 (s, 3H, H-10), 3.71 – 3.62 (m, 1H, H-9), 2.97 (dd, *J* = 13.8, 6.0 Hz, 1H, H-7'), 2.74 (dd, *J* = 13.8, 7.1 Hz, 1H, H-7), 1.46 (s, 3H, H-19), 1.38 (s, 3H, H-20). **^{13}C NMR APT** (101 MHz, $CDCl_3$) δ

(ppm) = 149.5 (C-1, C qt.), 146.9 (C-2, C qt.), 137.3 (C-12, C qt.), 130.7 (C-4, C qt.), 128.55 (C-16, C-14, CH), 127.81 (C-15, CH), 127.28 (C-17, C-13, CH), 121.1 (C-5, CH), 114.1 (C-3, CH), 113.0 (C-6, CH), 109.1 (C-18, C qt.), 76.7 (C-8, CH), 71.1 (C-11, CH_2), 68.95 (C-9, CH_2), 55.99 (C-10, CH_3), 39.66 (C-7, CH_2), 27.06 (C-19, CH_3), 25.75 (C-20, CH_3). HRMS-ESI (*m/z*) $[M+ H]^+$, calcld. for $C_{29}H_{24}O_4$, 328.1675; found 328.1669.

2.8 General procedure for obtention of nitrobenzoate derivatives (10a-c) by Steglich Esterification:

In a 10 mL test tube, 100 mg (1 eq.) of 2-nitrobenzoic acid was added 184 mg (1.6 eq.) of EDC and 8 mg of DMAP in 1 mL of dried dichloromethane. Then, 0.28 mL (2 eq.) of the suitable alcohol was added and the reaction was kept under stirring for 12 h at room temperature. After completion time, the reaction mixture was diluted in 20 mL of ethyl acetate and washed with 10 mL of 0.1 M aqueous HCl solution (1x), 10 mL of 10% aqueous $NaHCO_3$ solution and 10 mL of water (2x). The organic layer was dried over anhydrous Na_2SO_4 and concentrated by rotary evaporation. The residue was purified by column chromatography with cyclohexane / ethyl acetate (9:1, v/v) as eluent to obtain the desired product. Conditions adapted from Neises (1978).

10a – ethyl 2-nitrobenzoate:

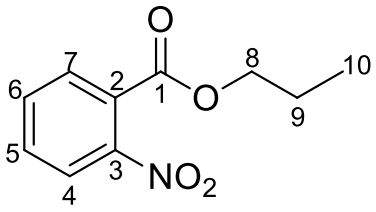


10a

Yellow Liquid. It was obtained using ethanol as the alcohol. Yield = 65.4% .**^{1}H NMR** (400 MHz, Chloroform-d) δ (ppm): 7.90 (dd, J = 7.9, 1.4 Hz, 1H, H-4), 7.74 (dd, J = 7.5, 1.7 Hz, 1H, H-7), 7.67 (td, J = 7.5, 1.5 Hz, 1H, H-5), 7.62 (td, J = 7.6, 1.7 Hz, 1H, H-6), 4.39 (q, J = 7.1 Hz, 2H, H-8), 1.35 (t, J = 7.2 Hz, 3H, H-9). **^{13}C NMR APT** (101 MHz,

CDCl3) δ (ppm): 165.41 (C-1, C qt.), 148.27 (C-3, C qt.), 132.90 (C-6, CH), 131.68 (C-5, CH), 129.86 (C-7, CH), 127.89 (C-2, C qt.), 123.88 (C-4, CH), 62.52 (C-8, CH_2), 13.80 (C-9, CH_3). HRMS-ESI (*m/z*) $[M+H]^+$, calcld. for $C_9H_9NO_4$, 195.0532; found 195.0526;

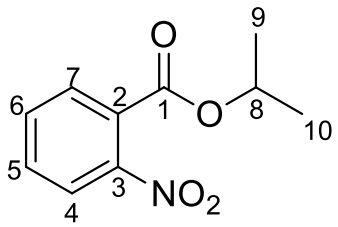
10b – propyl 2-nitrobenzoate:



10b

Yellow Liquid. It was obtained using propanol as the alcohol. Yield = 60%. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ (ppm): δ 7.91 (dd, *J* = 7.8, 1.5 Hz, 1H, H-4), 7.77 (dd, *J* = 7.5, 1.8 Hz, 1H, H-7), 7.69 (td, *J* = 7.5, 1.5 Hz, 1H, H-5), 7.64 (td, *J* = 7.6, 1.8 Hz, 1H, H-6), 4.31 (t, *J* = 6.7 Hz, 2H, H-8), 1.77 (h, *J* = 7.2 Hz, 2H, H-9), 1.00 (t, *J* = 7.4 Hz, 3H, H-10). **^{13}C NMR APT** (101 MHz, $CDCl_3$) δ 165.5 (C-1, C qt.), 132.8 (C-6, CH), 131.7 (C-5, CH), 129.9 (C-7, CH), 127.8 (C-2, C qt.), 123.8 (C-4, CH), 68.1 (C-8, CH_2), 21.7 (C-9, CH_2), 10.4 (C-10, CH_3). HRMS-ESI (*m/z*) $[M+H]^+$, calcld. for $C_{10}H_{11}NO_4$, 209.0688; found 209.0679;

10c- isopropyl 2-nitrobenzoate:



10c

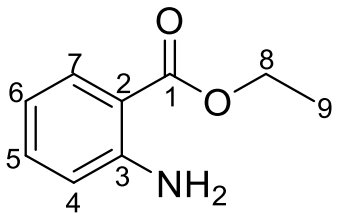
Yellow Liquid. It was obtained using isopropanol as the alcohol. Yield = 36%. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ (ppm): 7.90 (d, *J* = 8.0 Hz, 1H, H-4), 7.74 (d, *J* = 7.3 Hz, 1H, H-7), 7.70 – 7.64 (m, 1H, H-5), 7.64 – 7.59 (m, 1H, H-6). 5.27 (p, *J* = 6.3 Hz, 1H,

H-8), 1.35 (d, *J* = 6.3 Hz, 6H, H-10, H-11). **^{13}C NMR APT** (101 MHz, $CDCl_3$) δ (ppm): 165.0 (C-1, C qt.), 148.2 (C-3, C qt.), 132.9 (C-6, CH), 131.5 (C-5, CH), 129.8 (C-7, CH), 128.2 (C-2, C qt.), 123.8 (C-4, CH), 70.5 (C-8, CH), 21.4 (C-9, C-10, CH_3). HRMS-ESI (*m/z*) $[M+H]^+$, calcld. for $C_{10}H_{11}NO_4$, 209.0688; found 209.0681.

2.9 General procedure for nitrobenzoate reduction to anthranilate esters (11a-c) by Tin (II) Chloride:

In a 50 mL reaction flask, 200 mg (1 eq.) of the nitrobenzoate esters obtained by Steglich esterification was added together with 800 mg (5 eq.) of Tin (II) Chloride and 30 mL of ethyl acetate. The reaction mixture was kept under stirring and reflux for 4 h. Upon completion, the reaction flask was cooled in an ice bath, and then 20 mL of ethyl acetate and 5 mL of water were added. In the reaction mixture, saturated sodium bicarbonate solution (10%, w/v) was added carefully until the pH was adjusted between 7-8. After that, the mixture was filtered in order to purify from the insoluble formed complex, then extracted with 20 mL of brine. The organic layer was dried over anhydrous Na_2SO_4 and then concentrated by rotary evaporation. No further purification was required. Conditions adapted from Bellamy (1984).

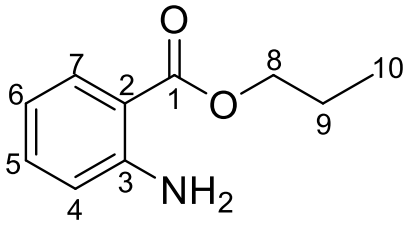
11a- ethyl 2-aminobenzoate:



11a

Light yellow liquid. Yield: 59%. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ (ppm): 7.92 – 7.83 (m, 1H, H-7), 7.30 – 7.20 (m, 1H, H-5), 6.65 (s, 1H, H-6), 6.64 – 6.60 (m, 1H, H-4), 5.71 (s, 2H, NH_2), 4.32 (q, *J* = 7.1 Hz, 2H, H-8), 1.37 (t, *J* = 7.1 Hz, 3H, H-9). **^{13}C NMR APT** (101 MHz, $CDCl_3$) δ 168.2 (C-1, C qt.), 150.5 (C-3, C qt.), 134.0 (C-5, CH), 131.2 (C-7, CH), 116.7 (C-6, CH), 116.2 (C-4, CH), 111.1 (C-2, C qt.), 60.3 (C-8, CH_2), 14.4 (C-9, CH_3). HRMS-ESI (*m/z*) $[M+H]^+$, calcld. for $C_9H_{11}NO_2$, 165.0790; found 165.0790;

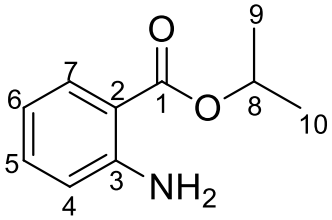
11b- propyl 2-aminobenzoate:



11b

Yellow Solid. Yield: 40%. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ (ppm): 7.91 (dd, *J* = 8.3, 1.7 Hz, 1H, H-7), 7.27 (ddd, *J* = 8.3, 7.1, 1.6 Hz, 1H, H-5), 6.65 – 6.62 (m, 1H, H-6), 6.61 (dd, J = 5.1, 1.2 Hz, 1H, H-4), 5.74 (s, 2H, NH_2), 4.27 (t, *J* = 6.6 Hz, 2H, H-8), 1.81 (h, *J* = 7.4 Hz, 2H, H-9), 1.06 (t, *J* = 7.4 Hz, 3H, H-10). **^{13}C NMR APT** (101 MHz, CDCl3) δ (ppm): 168.2 (C-1, C qt.), 150.5 (C-3, C qt.), 133.9 (C-5, CH), 131.2 (C-7, CH), 116.7 (C-6, CH), 116.2 (C-4, CH), 111.2 (C-2, C qt.), 65.9 (C-8, CH_2), 22.2 (C-9, CH_2), 10.5 (C-10, CH_3). HRMS-ESI (*m/z*) $[M+ H]^+$, calcld. for $C_{10}H_{13}NO_2$, 179.0946; found 179.0947;

11c- isopropyl 2-aminobenzoate:



11c

Yellow Solid. Yield: 33%. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 7.87 (ddd, *J* = 7.8, 1.6, 0.7 Hz, 1H, H-7), 7.28 – 7.22 (m, 1H, H-5), 6.66 (d, J = 0.7 Hz, 1H, H-6), 6.64 – 6.60 (m, 1H, H-4), 5.71 (s, 2H, NH_2), 5.21 (p, *J* = 6.3 Hz, 1H, H-8), 1.36 (s, 3H, H-9), 1.35 (s, 3H, H-10). **^{13}C NMR APT** (101 MHz, $CDCl_3$) δ (ppm): 167.7 (C-1, C qt.), 150.4 (C-3, C qt.), 133.9 (C-5, CH), 131.3 (C-7, CH), 116.7 (C-6, CH), 116.2 (C-4, CH), 111.5

(C-2, C qt.), 67.6 (C-8, CH), 29.7 (C-9, CH_3), 22.0 (C-10, CH_3). HRMS-ESI (*m/z*) $[M+H]^+$, calcld. for $C_{10}H_{13}NO_2$, 179.0946; found 179.0948.

2.10 GENERAL METHODOLOGY FOR REPELLENCY TESTS

2.11 Mosquito breeding

Aedes aegypti females from the colony of the Insect Bioecology Laboratory (LaBEIn) of the Federal University of Rondônia (Unir) were used for the experiment.

The eggs obtained from the females were placed in plastic bowls (30 x 22 x 8 cm) containing one liter of water for larval hatching. Larvae belonging to the first two stages (L1 and L2) received daily feed of ground fish (TetraMin® Tropical flakes). From the third stage (L3) these larvae were transferred to plastic containers (2L), with screens, containing one liter of water and fed with Reptolife® (BARBOSA et al. 2019).

The larvae remained in these containers until pupae and later adults, and were transferred to screened cages. They were fed daily with cotton wool soaked with 10% sucrose solution, kept under controlled laboratory conditions of temperature between 26-27ºC, humidity 70-80% and photoperiod of 12 hours.

2.12 Sting Protection Experiments

The module used in the bioassays was adapted from a 0.48L (11.5 x 10 x 7.5 cm) polypropylene container with a snap cap (Plasvale®). The adaptation was made through two holes: (i) side of the container (0.5 cm radius), for insertion and removal of mosquitoes, being sealed with satin vinyl foam (EVA) to prevent specimen leakage; (ii) upper part (1.5 cm radius) for mosquito access to the impregnated organza screen (12.56 cm2) supported by an EVA edge to prevent mesh / container contact (Figure 2A).

2.13 Bioassays

The tests were divided into the following experimental groups: (i) Treatments of the tested compounds **1-10** (diluted in Ethanol POH); (ii) positive control (N, N-Diethyl-m-toluamide - DEET) all at a concentration of 200 nmoles/cm2 and (iii) negative control

(P.A. Ethyl alcohol). After dilution, the substances were applied to a micropipette mesh with a drying period of 10 minutes.

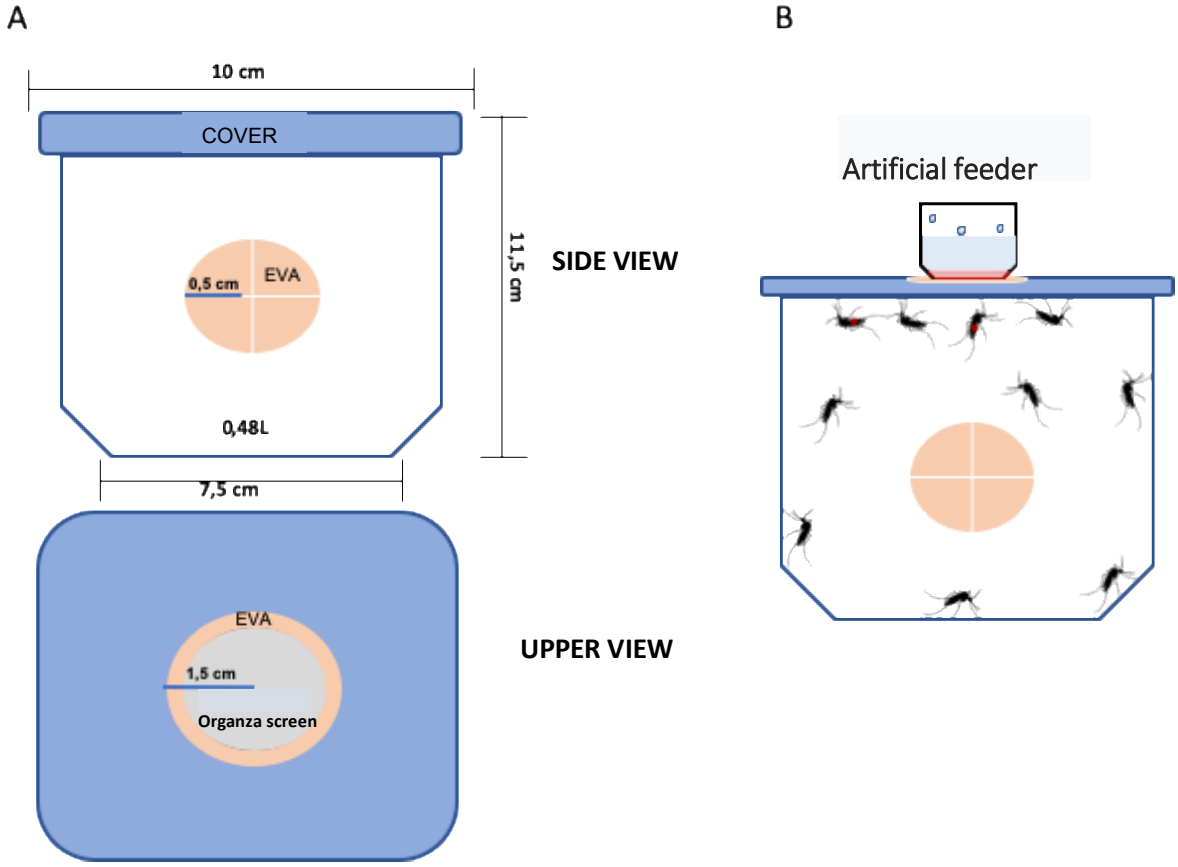


Figure 2. Experimental module for experiments to evaluate bite protection substances. (A) Dimensions and main features in two views; (B) Scheme of the experimental module with artificial feeder and mosquitoes. Source: Amanda Santos, 2019.

Nulliparous females from Ae. aegypti at 5 to 10 days of age were separated and fasted for 36 hours before the experiment. 10 females per replicate (n = 8) were used in 3 replications, with in total 1760 females.

With the module prepared, the females were inserted with the aid of a Castro trap and acclimatized for 5 minutes, after which time they were exposed to the compounds using an artificial feeder adapted from SÍRIA *et al.* (2018) used for feeding mosquitoes and as a source of attraction by simulating a vertebrate host (Figure 2B).

Following exposure of mosquitoes to the compounds, the observation and recording of the number of landings per minute for 5 minutes began. At the end of exposure time, the number of engorged mosquitoes was recorded to estimate the number of bites.

The bite protection index (P) was calculated according to the formula P = (CT) / C, where C is the number of engorged mosquitoes in the negative control, i.e. solvent-treated tissue and T is the number of engorged mosquitoes. Treatment, i.e., tissue treated with the evaluated substances (WHO, 2009)

The number of landings over time was evaluated by two-way ANOVA (time x substance) and comparisons were made by Dunnet's test. The number of landings over the total observation time was analyzed by the Kruskal-Wallis test, comparisons by the Dunn test and the number of engorged mosquitoes were analyzed by one-way Anova, after the observation time. Comparisons were made by Tukey test. All tests were performed using the Prism 8 program (Graph Pad Inc) and the significance level used was 5%.

3 RESULTS AND DISCUSSION

3.1 Synthesis

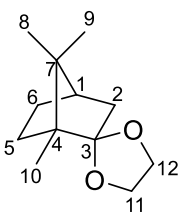
Herein 23 compounds were synthesized as showed in Figure 3. The structure of the compounds was confirmed by NMR and MS data which agree with the proposed structures. The compounds were designed in order to have a structural diversity based in active chemical classes in the literature regarding repellent activity. The series of compounds were organized per scaffold as: 1) series of camphor; 2); series of L-menthone 3) series of D-limonene 4) series of eugenol and 5) series of anthranilic acid.

The functional group transformations were achieved using the following reactions type: Acetal formation, Baeyer-Villiger oxidation, Sharpless asymmetric dihydroxylation, Steglich esterification and Nitro reduction. The synthetic routes were presented in Scheme 2, 3 and 4.

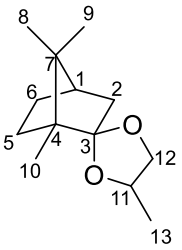
Limonene and eugenol (protected by methylation), natural repellents, were converted to diols, an important functional group for repellency (POHLUCH *et al.*, 2010, XUE *et al.*, 2007). Acetal synthesis for all compounds is required to reduce volatility according literature (IOVINELLA *et al.*, 2014). D-camphor and L-menthone were converted to lactones in order to study the influence of this moiety as esters can increase the repellent efficiency (NESTERKINA *et al.*, 2018). Synthesis of eugenol ethers were carried off and these derivatives were never evaluated for repellency but have larvicide activity (BARBOSA *et al.*, 2012). Reduction of the nitro group to amine was obtained to afford anthranilate esters, well-known repellents with renewed interest in literature (ISLAM, 2017; ZHAO *et al.*, 2019). The discussion of the synthesis was presented based in reaction similarity and in the order presented in Figure 3.

3.1.1 Compounds synthesized in this work

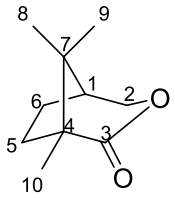
D-Camphor Series



1a

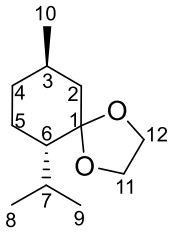


1b

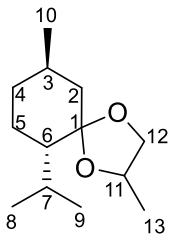


2

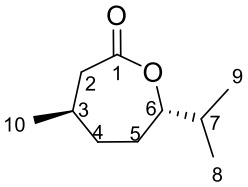
L-Menthone Series



3a

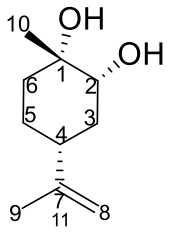


3b

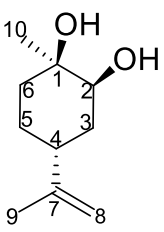


4

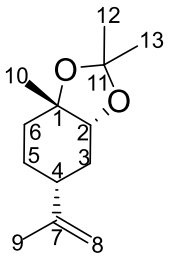
D-Limonene Series



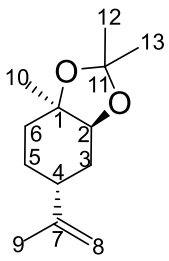
5a



5b



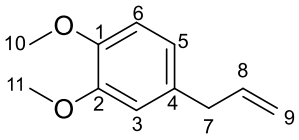
6a



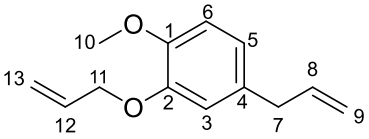
6b

Figure 3. Compounds synthesized in this work organized by their respective series.

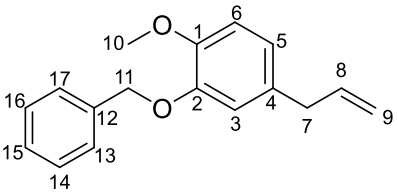
Eugenol series



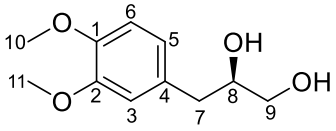
7a



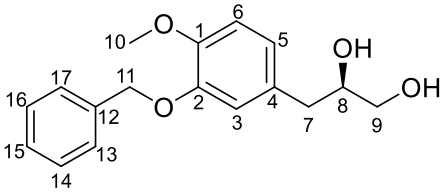
7b



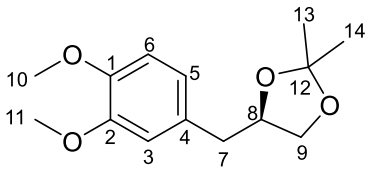
7c



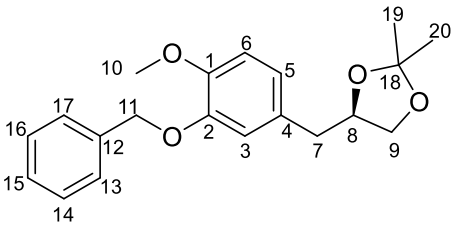
8a



8b

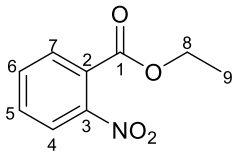


9a

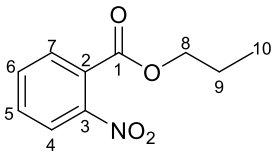


9b

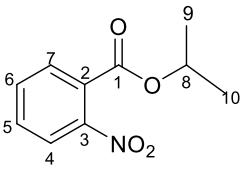
Nitro benzoate series



10a



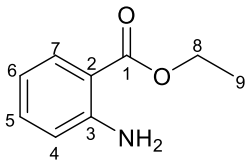
10b



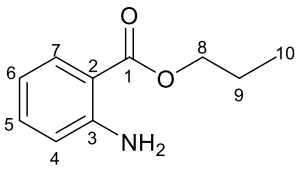
10c

Figure 3 (cont.). Compounds synthesized in this work organized by their respective series.

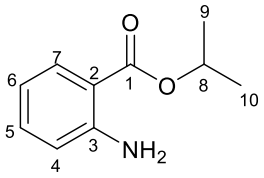
Anthranilate series



11a



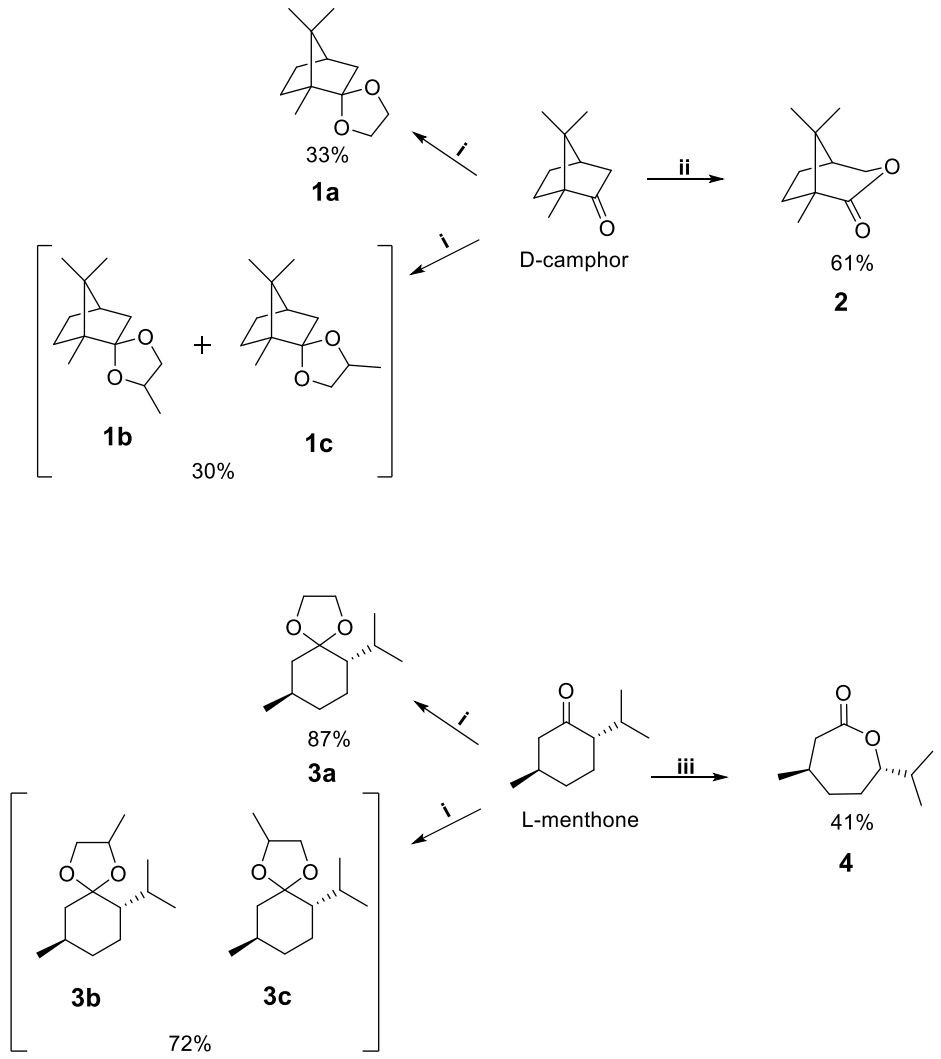
11b



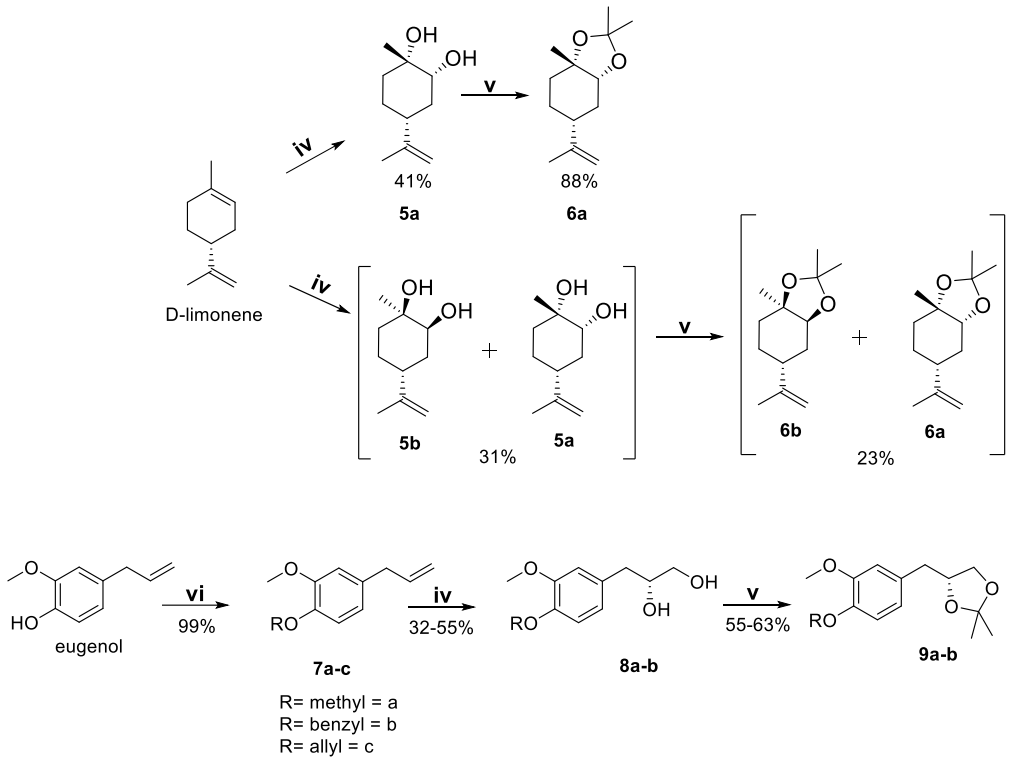
11c

Figure 3 (cont.). Compounds synthesized in this work organized by their respective series.

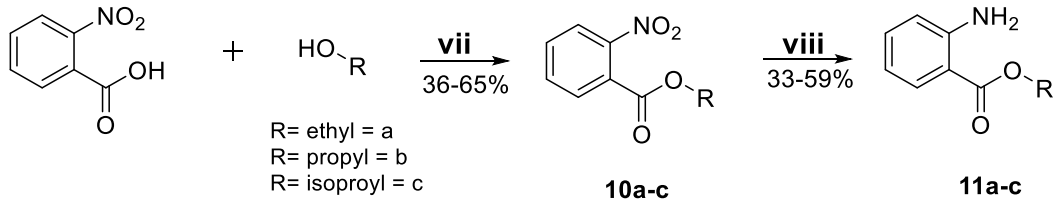
3.1.2 Synthetic routes used to obtained the compounds



Scheme 1. Preparation of D-camphor and L-menthone acetals and lactones. Reagents and conditions: **i**-TsOH, ethylene glycol (**1a**) and propylene glycol (**1b**), toluene, reflux, 48 h; **ii**- m-CPBA, TfOH, r.t, 3 h; **iii**- m-CPBA, TsOH, CH_2Cl_2, reflux, 24 h. Compounds **1b** and **3b** were obtained as a mixture of four diasterisomers; Compound **3a** was obtained as a mixture with isomenthone.



Scheme 2. Preparation of D-limonene and eugenol diols and acetals. Reagents and conditions: **iv**- $K_2OsO_4.2H_2O$, $DHQD_2PHAL$ (for 5a), DHQ_2PHAL (for 5b), $K_3Fe(CN)_6$, K_2CO_3, t-BuOH/H_2O, 0 ºC, 16 h; **v**- 2,2-dimethoxypropane, $BF_3.Et_2O$, 8 h; **vi**- K_2CO_3, appropriate alkyl chloride, DMF, reflux, overnight; Compound **5b** was obtained as a mixture with **5a**. Compound **6b** was obtained as mixture with **6a**.



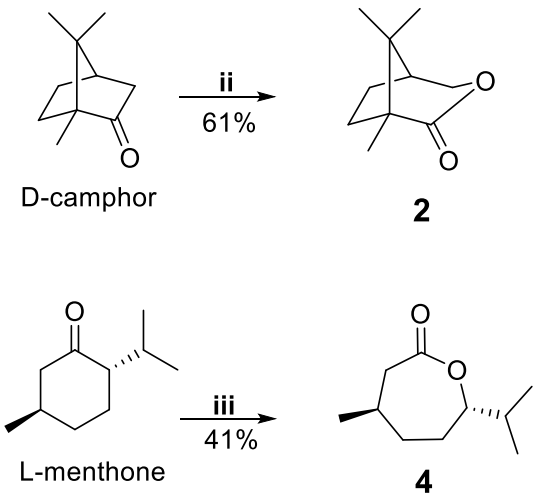
Scheme 3. Preparation of nitrobenzoates and anthranilate esters. Reagents and conditions: **vii**- EDC, DMAP, appropriate alcohol, 25 ºC, 16 h; **viii** – $SnCl_2$, EtOH, 60 ºC, 4 h.

3.2 Synthesis of lactone derivatives

3.2.1 Baeyer-Villiger Oxidation of camphor and menthone to obtain 2 and 4

D-camphor was submitted to Baeyer-Villiger oxidation (BVO) using m-CPBA as oxidizing agent and triflic acid (TfOH) as the catalyst. The reaction was completed in 3 h at room temperature to afford **2** with final yield of 61%. The same reaction was applied to L-menthone but using TsOH as catalyst and a longer reaction time (24 h) in reflux. The final yield of **4** was 41%.

The formation of the lactone was confirmed by signals around δ 170-180 ppm in the ^{13}C NMR spectra, which were attributed to an ester group in comparison with the ketone shift around δ 200 ppm of the starting materials. Both lactones were fully characterized by ^{1}H, ^{13}C NMR and HRMS which data correspond to those presented by Adolph Baeyer and Victor Villiger whom first reported these lactones (BAEYER and VILLIGER, 1899).



Scheme 4. Preparation of D-camphor and L-menthone lactones. Reagents and conditions: **ii**- m-CPBA, TfOH, r.t, 3 h; **iii**- m-CPBA, TsOH, CH_2Cl_2, reflux, 24 h.

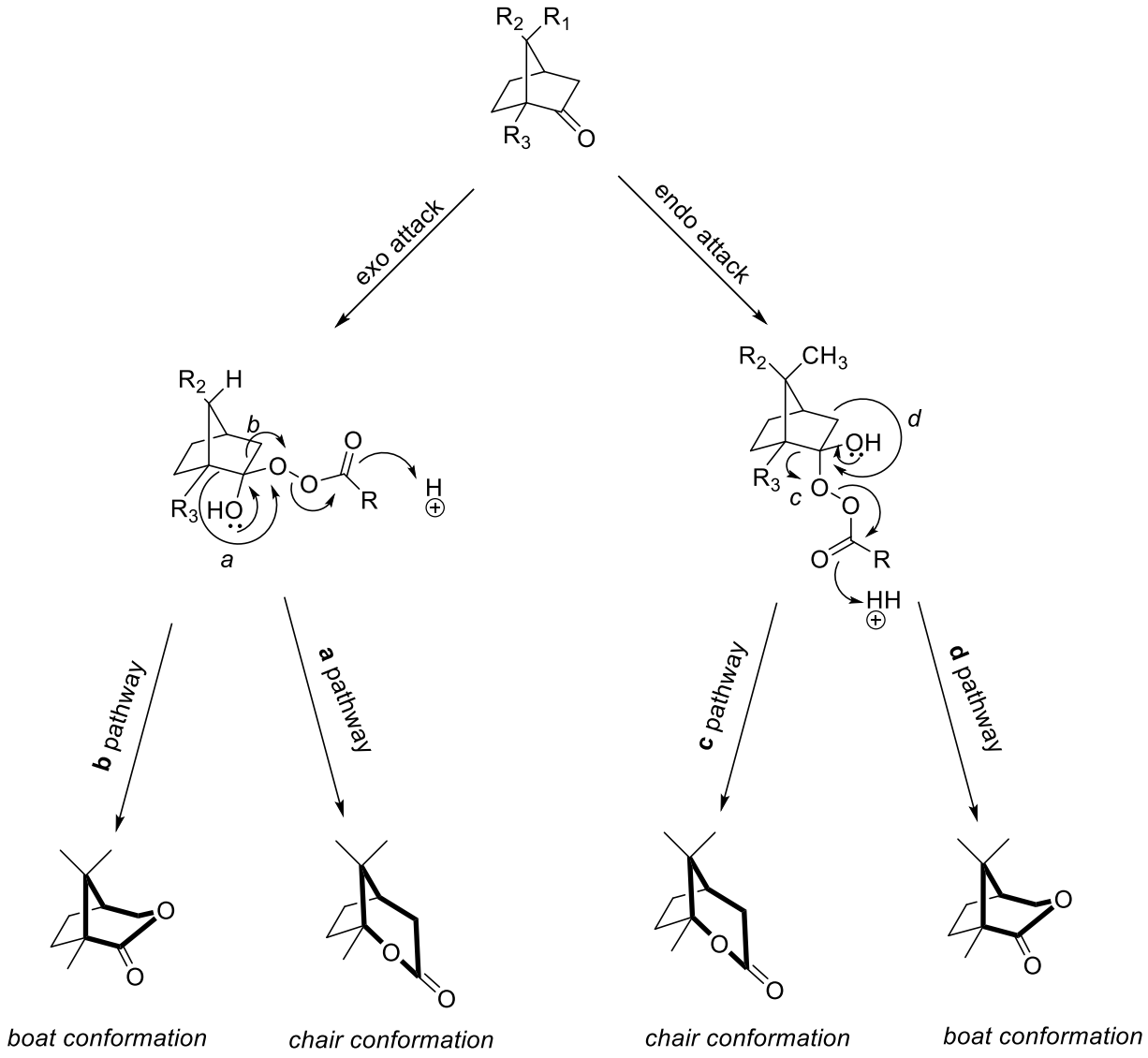
Herein, the Baeyer-Villiger reaction with mCPBA catalyzed by TsOH was applied as the first attempt for the D-camphor. No product was formed, remaining only the starting material. This result was also observed by Forest (2003) in his thesis of camphor auxiliaries, and in order to obtain the final product, the author used the stronger acid TfOH as catalyst and thus, only one regioisomer was obtained. The same conditions were reproduced herein with the same outcome reported by Forest (2003): a single compound was obtained.

In relation to the reaction mechanism, it caused a debate between the 50's to 60's in regards of which products were formed when using bicyclic compounds like camphor. In 1958, a reasonable mechanism was proposed to predict the final regioisomers. This mechanism was based in the competition between steric and electronic effects, with distinct *exo* and *endo* peracid attacks and carbon shifts, with different final products possessing boat or chair conformation (Scheme 6) (MURRAY *et al.*,1956) When applying TfOH, only pathway *d* happens, leading only to regioisomer **2**. One of the reasons may be due to the very strong triflic acid, which could lead to the carbonyl protonation (HAWTHORNE and EMMONS, 1958), and favor the *d* pathway.

Considering our NMR data, and those from Forest (2003) with a similar compound, it was possible to prove the formation of **2**, as Fig. A12 in Supplementary Material. In the ^{13}C NMR spectrum of **2**, the presence of one carbon at δ 73.9 was attributed to C-2, next to oxygen, which is present in the boat conformation. This chemical shift is only present in this regioisomer and complete absent in the chair conformation. It is very important to mention, that in our work we could attribute in detail all the NMR signals for this compound. Besides the boat conformation, Meinwald and Frauenglass (1960) identified the chair conformation in norcamphor, in agreement with the model by Murray (1956). The chair conformation is also present in the work of Forest (2003) in ^{13}C NMR data of a similar compound, with clearly differences in shifts between boat and chair conformation.

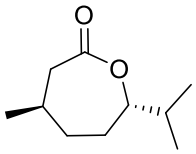
This single regioisomer product (**2**) suggested a preferred mechanism by steric effects and the formation of one regioisomer by *endo* attack, with the pathway *d,* as presented in Scheme 5. The steric effects are due to the presence of the α-methyl group in the bridgehead of camphor. If no methyl is present (for example norcamphor), then, the mechanism will follow by *exo* attack by the oxidizing agent.

Therefore, the formation of **2** fits in the model of Hawthorne and explain the results in this Baeyer-Villiger oxidation. In considerations of practical work, the synthesis applying TfOH was fast (3 h) with no extreme or harsh conditions (room temperature) and the yield is good (61%). Only downsizes it is manipulating the triflic acid, one of the strongest acids ever known, that can lead to accidents if misuse.



Scheme 5. The formation mechanism of the lactone products of D-camphor by *exo* and *endo* approach and the conformation of the final products. Adapted from Renz and Meunier (1999).

Then L- menthone was submitted to Baeyer-Villiger oxidation, applying the reagents TsOH/mCPBA in the first place instead of triflic acid. In contrast with D-camphor, the reaction proceeded without problems and with the formation of only one compound, the lactone **4**. Differently from the mechanism of camphor, the outcome for L-menthone may be explained by the ability of the carbon migration. Usually the oxygen insertion is favored if the carbon is tertiary, and in this case the oxygen is inserted next to the carbon where isopropyl is attached, thus stabilizing the δ+ charge (DOERING and SPEERS, 1950). The structure was confirmed by our NMR data (Supplementary Material, Figure S18). In ^{13}C NMR and APT spectra, the signal at δ 84.5 was attributed to CH-6 next to oxygen, neighbor of carbonyl moiety, being more deshielded, leaving no doubts in the formation of this lactone. Bayer and Villiger (1899) first reported this lactone.



4

Figure 4. Structure of L-menthone lactone 4

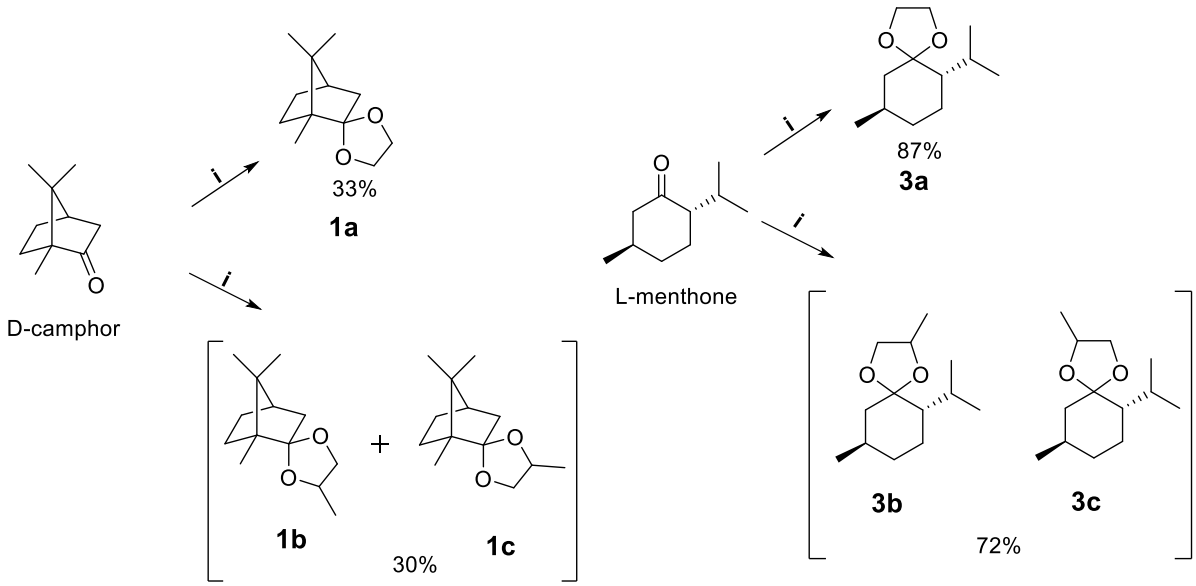
The use of a mild acid in comparison with TfOH made this reaction more affable for L-menthone than D-camphor. On the other hand, it takes more time and the use of dried solvents and reflux, with lower yield (41%) than the oxidation of camphor (61%). In general, the reaction occurred with other catalysts and acceptable yields. By following a mechanism model presented in Scheme 5, the final products can be predicted. Beyond conditions, the scaffold structure also has a big influence in the reaction. In addition, BVO operates with retention of configuration (RENZ and MEUNIER, 1999) being useful for asymmetric synthesis routes.

3.2.2. Acetal formation with D-Camphor and L-Menthone to obtain 1a-1b and 3a-b

The formation of acetals using D-camphor with ethylene (**1a**) or propylene glycol (**1b/1c**) and TsOH as catalyst in reflux was acquired with regular yields of 33% and 30%, respectively. Nomura and colleagues (1996) first reported the synthesis of D-

camphor acetal **1a** in 1996, while for **1b/1c** the only record comes from Sanz-Burrata *et al.* (1970). In our work, a full NMR attribution of **1a** is described for the first time, including 2D data.

Using L-menthone, the reaction presented the yields of 87% for the acetal coming from the ethylene glycol (**3b**) and 72% from the propylene glycol (**3a**). For L-menthone, Arterburn and Perry (1999) reported the acetal **3a** synthesis for the first time, and acetal mixture **3b/3c** was reported by Iovinella, 2014. None of the camphor derivatives were evaluated for repellent and larvicide activities.



Scheme 6. Preparation of D-camphor and L-menthone acetals. Reagents and conditions: **i**-TsOH, ethylene glycol (1a) and propylene glycol (1b/1c), toluene, reflux, 48 h. Compound **1b** and **3b** is obtained with **1c** and **3c**, each with a pair diasterisomers. Compound **3a** is obtained as a mixture with Isomenthone.

For the acetal formation of D-camphor and L-menthone, a proper diol was required. At first, the use of TfOH for the D-camphor scaffold and ethylene glycol, motivated by the fast reaction in BVO, was applied in a reflux system with Dean Stark apparatus for continuous water removing (CARON, 2011). After one hour, the reaction turned from a white opaque color to a dark solution. By monitoring the reaction by TLC,

numerous spots appeared indicating a possible fragmentation of the starting material. In this case TfOH with reflux presented no advantage for the formation of acetals, being preferable the use of TsOH, a classical catalyst, in reflux conditions.

The reaction completion time using the D-camphor and TsOH and ethylene glycol as catalyst was monitored by TLC and lasted for 48 h with the full consumption of the starting materials. This long reaction time may be caused by the low reactivity of the scaffold, being a bicyclic molecule with a considerable steric hindrance, even using diol small molecules. Moreover, this explanation could be used to the regular yields of 33% and 30% obtained for **1a** and mixture **1b/1c**, respectively. However, Nomura in 1996 using D-camphor, ethylene glycol and TsOH obtained **1a** with 70% yield, but with longer reaction time (72 h). Although this study is the only reference for this acetal, it did not present any detailed data, and a poorly NMR description of **1a** without any detailed attribution of the signals. A full description of all signals and 2D attributions is present in Supplementary Material, being to date, the first time that **1a** is fully resolved in the literature.

For the correct attribution of all signals, besides the ^{1}H NMR and ^{13}C APT, the 2D experiments were fundamental. Although the ^{1}H-^{1}H COSY (Figure S4) presented the neighbor protons correctly, clarifying that H5 and H6 relationship and especially in the case of H2 that is next to the single proton of H1, the main issue is the right position for the H2, H5 and H6 inside the six-member ring of this bicycle molecule. Even when ^{1}H-^{13}C-HSQC (Figure S5) is available and in conjunct with APT, it was not clear. However, the HSQC experiment was important to attribute C1 correctly, because it is a CH and the data provided leaves no doubt. In addition, it gives us information of C-2, C-5 and C-6 being CH2, and in the case of C-2 being next to acetal group, where the electronegative groups are present, making this carbon more deshielded (δ 44.6), and in agreement what is observed in HSQC. Yet, the mystery was not solved, and ^{1}H-^{13}C-HMBC (Figure S8). was necessary to clarify the correct positions of the presumed C-5 and C-6. In the spectra, the shifts of the protons of C-10, C-8 and C-9 were crucial to do the correct attributions. As HMBC presents the long-range coupling of protons and two and in some cases, three carbons apart, C-2 is the main choice to start. As -C2 can only couple with H8 and H9, because H10 is at a distance of four carbons apart, two signals were expected in HMBC. Based in the C-2 information in ^{1}H-^{13}C-HSQC, with C-2 being more deshielded the coupling with H8 and H9 was observed

with two signals in the spectra, resolving the position of C-2. This leads us for the final attribution of C-5 and C-6. For C-6, no correlation was found, but C-5 (CH2), presents a correlation with H10. This correlation can only happens if C-5 is in the guessed position. In addition, the HMBC spectra clearly presents the relationships of the quaternary carbons C-4 and C-7 with the neighbor protons of the methyl groups H8, H9 and H10. With all the correlations resolved without any doubts, C-6 was the only CH2 carbon left. It is attribution was fully resolved based in the protons of H6, because C-5 and C-1 were already resolved. In the COSY spectra, it shows that H6 and H6' are neighbors of H5 and H6, leaving no question in it is position in the ring. With all of these experiments and data, a correct attribution of **1a** was made for the first time.

In regards of products, a single product was obtained when working with ethylene glycol. The NMR data of **1a** is in agreement with the structure. Changing the diol for propylene glycol, it is likely that a mixture with **1b** and **1c**, with a pair of diasterisomers each will be formed. This is true as presented in the study of Iovinela (2014) with menthone, and the same is observed herein with mixture **1b/1c,** with a clearly quadruplicated signal at δ around 117 ppm attributed to the quaternary carbon C-1 of acetal moiety.

When changing the scaffold to L-menthone, the yields of acetal formation with both diols were two times higher in comparison with camphor. Only one compound is expected when working with ethylene glycol but a mixture was observed for **3a** in ^{13}C NMR. Initially, because the starting material is L-menthone 96%, possessing a mixture of isomers as impurity, that result may be explained as possible contamination of the starting material. In order to verify this hypothesis, L-menthone was purified by CC and characterized by NMR. The presence of only one compound was identified according the ^{13}C NMR data in Figure 5. Repeating the acetal reaction with this pure L-menthone, **3a** was obtained again as a mixture, which was confirmed by CG-MS and NMR data as in Supplementary Material (Figure S15 and S16) and in Figure 6. This latter result suggested that for menthone, acetal reactions may lead to epimerization. The epimerization occurs at carbon C-10, where methyl is present.

In the literature, this phenomenon was also observed by the works of Arterburn and Perry (1999), where the acetalization of L-menthone had led to epimerization, with a major L-menthone derivative and other minor isomenthone derivative. In addition, a

study conducted by Schneider and Viljoen (2002) in the synthesis of diosphenols, identified isomenthone as an optical impurity in menthone derivatives, even when starting with pure menthone. Years later, Skakovskii *et al.* (2010) reported the same observation when characterizing the essential oil from *Agastache rugosa,* where menthone was also presented as a mixture with isomenthone, being this last one a minor constituent. These evidences along with our experimental data supported that the reaction of L-menthone with ethylene glycol leads to epimerization.

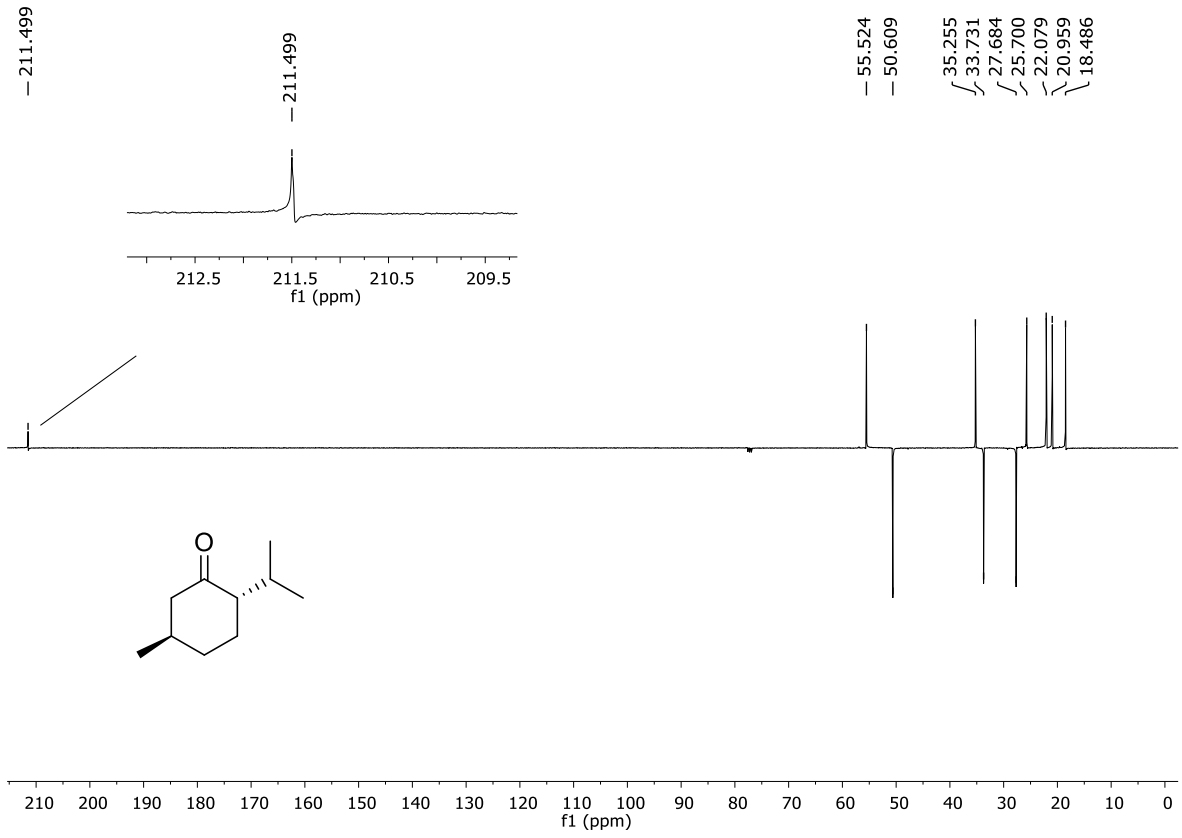


Figure 5. Purified L-menthone in ^{13}C NMR APT spectrum (400 MHz, $CDCl_3$)

When changing the ethylene glycol to propylene glycol the identification of the final products was very difficult because it was obtained the four expected diasterisomers due to the methyl group, but also, the formation of the minor isomenthone diasterisomers, with a total of 8 isomers. This is observed by the acetal shift of δ 110 ppm in ^{13}C NMR spectrum of this final product, in the Figure 7.

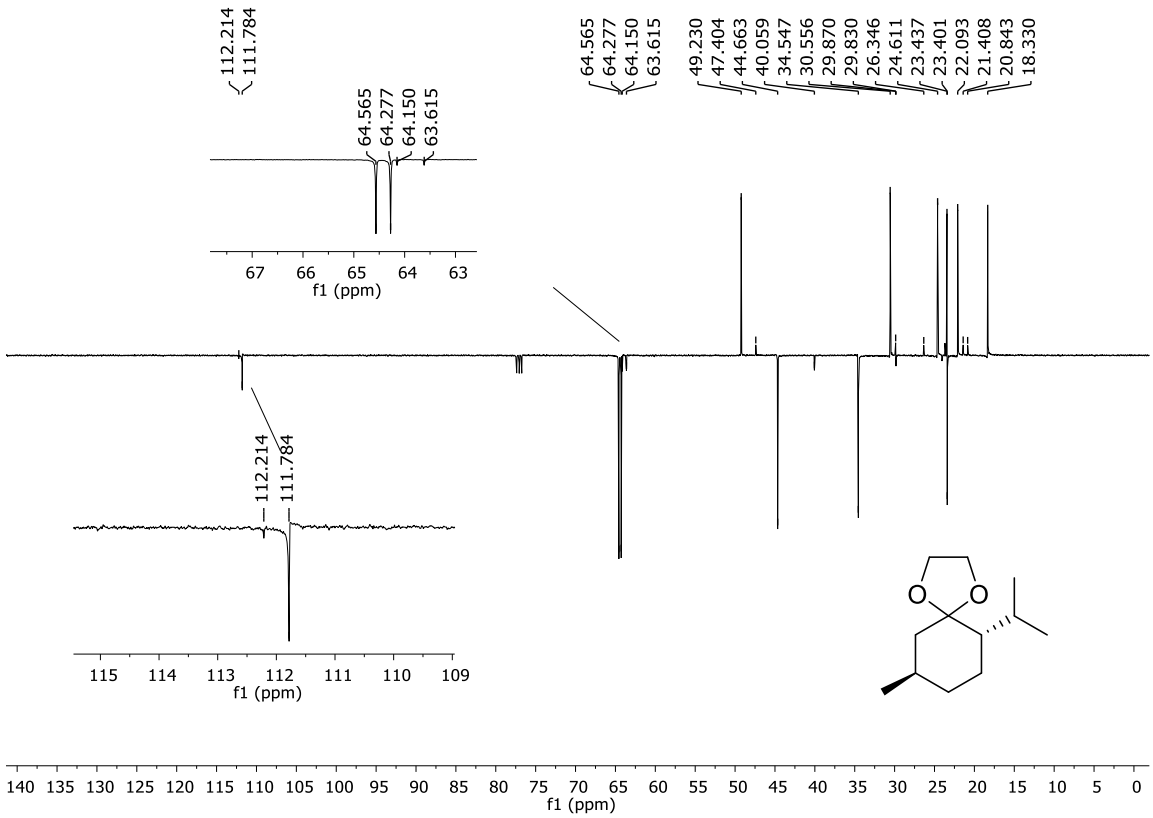


Figure 6. ^{13}CNMR APT spectra (400 MHz, $CDCl_3$) of **3a**, demonstrating the formation of a mixture as the final product. Acetal reactions with L-menthone led to epimerization

In both cases of D-camphor and L-menthone, the formation of acetal was possible but presented some struggles, mostly, because of the intrinsic nature of these scaffolds. D- camphor, being sterically hindered, presented low yields even with longer reaction time. In the other side, L-menthone converting itself partially to isomenthone can lead to diasterisomer mixture that is highly difficult to identify especially in the case of propylene glycol. Yet, the reactions were reproducible with formation of mixtures in all cases with exception of **1a**. These results agree with the literature.

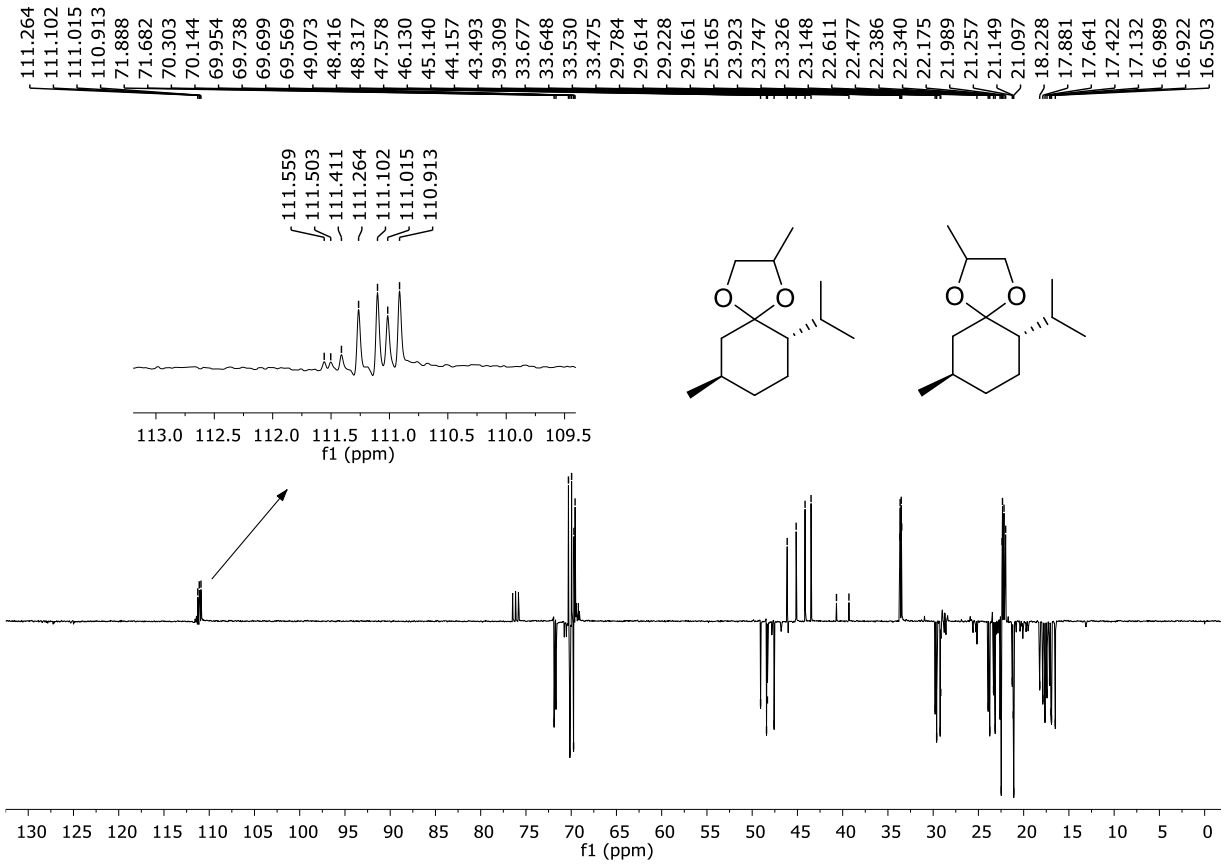


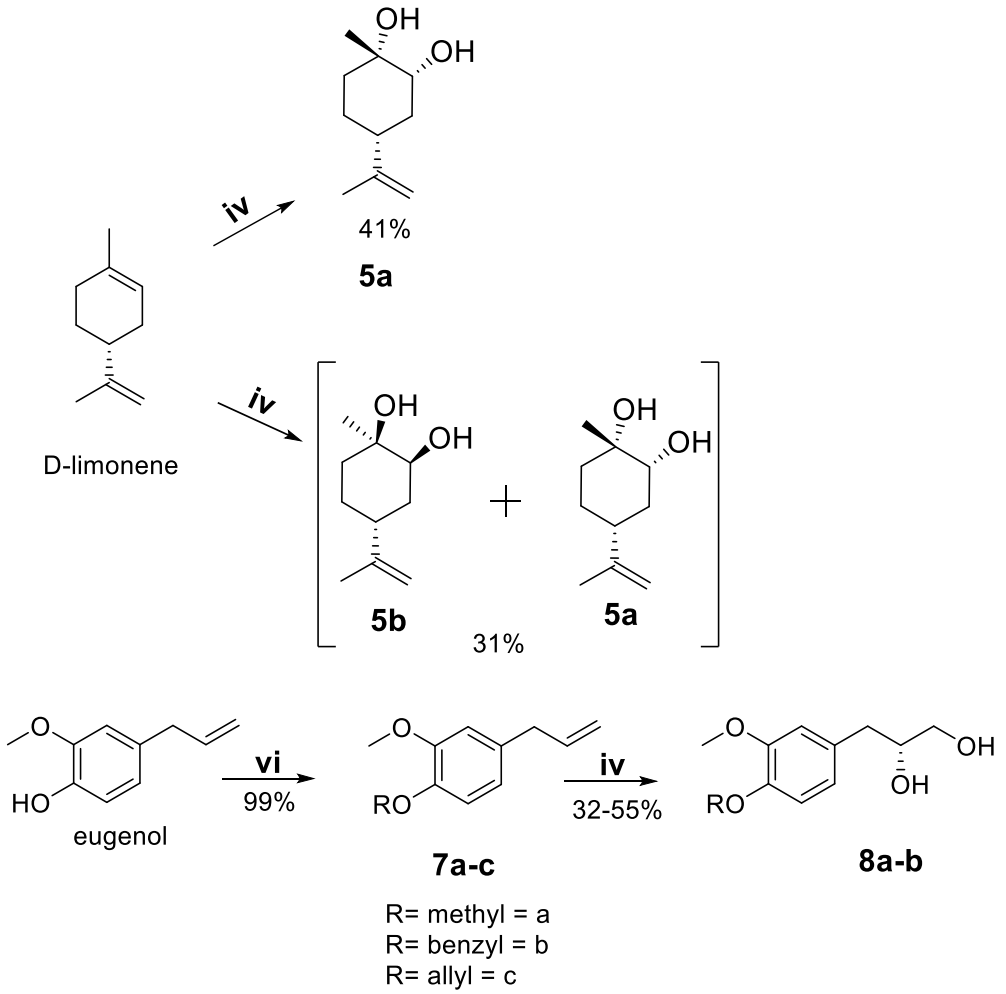
Figure 7. ^{13}CNMR spectra (400 MHz, $CDCl_3$) of mixture **3b/3c**, showing the formation of a mixture of isomers and diastereomers with L-menthone and propylene glycol.

3.3 The Sharpless Asymmetric Dihydroxylation and the formation of acetonide with eugenol and R- (+)- Limonene

Asymmetric synthesis was applied in this work to obtain products with specific stereochemistry, circumventing the formation of mixtures and posterior problems with isolation. Sharpless asymmetric dihydroxylation is a reliable reaction to induce the formation of asymmetric 1,2 diols and takes place in the olefin moiety presented in eugenol and limonene. Moreover, the asymmetric compounds will be useful to determine the importance of stereochemistry in SAR studies.

So, limonene and the eugenol ether derivatives were submitted to Sharpless asymmetric dihydroxylation.

Prior to formation of asymmetric diols, the eugenol derivatives were obtained by Williamson-ether synthesis. In this sense, the reaction using eugenol, K_2CO_3 and alkyl chlorides led to the formation of the eugenol ethers (**7a-c**) with 99% yield. One of the main reasons to alkylate the free hydroxyl of eugenol is to prevent potential side reactions. Moreover, eugenol ethers presented better larvicide activities (BARBOSA *et al.*, 2012).

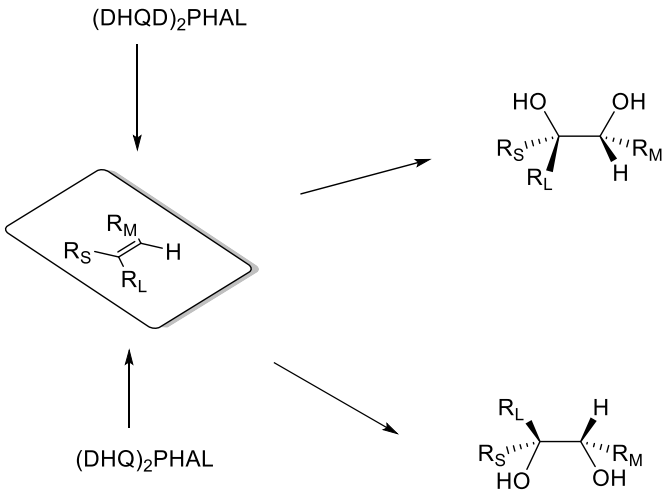


Scheme 7. Preparation of D-limonene and eugenol diols and acetals. Reagents and conditions: **iv**- $K_2OsO_4.2H_2O$, $DHQD_2PHAL$ (for 5a and 8a), DHQ_2PHAL (for 5b), $K_3Fe(CN)_6$, K_2CO_3, t-BuOH/H_2O, 0 ºC, 16 h; **vi**- K_2CO_3, appropriate alkyl chloride, DMF, reflux, overnight. Derivative **5b** is obtained as a mixture with **5a**.

The Sharpless reaction using K_2OsO_4 $2H_2O$, $DHQD_2$ PHAL (AD-mix- β) and K_3Fe [CN_6] went smoothly in 16 h at 0º C with a 41% yield after purification for **5a**, 31%

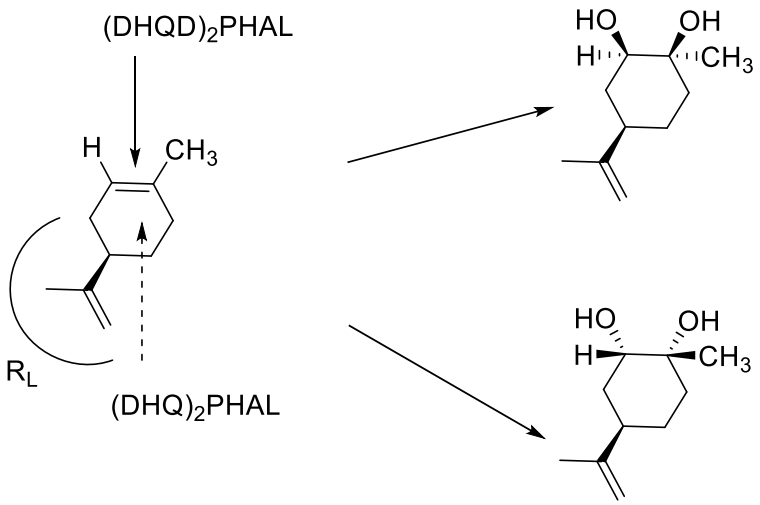
for **5b**, 32% for **8a**, and 55% to **8b**. All the derivatives were fully identified by NMR data with proper attribution of signals and stereochemistry. For asymmetric diols of eugenol derivatives, the only novel compound is **8b**. The diol **8a** was first described by Sawant and Waghmode (2010) using L-proline with chiral pool. In our work, it is the first time these eugenol derivatives were obtained by Sharpless asymmetric dihydroxylation. These limonene diols were first reported by Hao *et al.* (2016), followed by Wang *et al.* in 2017, then appearing again in the literature in a study of Morikawa *et al.* (2019).

A simple model predicts the final stereochemistry of the products according Kolb *et al.* (1994). In Scheme 8, the possible products and dihydroxyl addition modes are presented.



Scheme 8. Addition of the hydroxyl groups according each AD-mix ($(DHQD)_2PHAL$ and $(DHQ)_2PHAL$) reagent used to afford the corresponding enantiomers. Adapted from Kolb, 1994.

Herein, it was tested the two AD-mix reagents: AD-Mix-β and AD-Mix-α. Initially the $(DHQD)_2PHAL$ corresponding to AD-Mix- β was first used. Using this catalyst, 1,2 limonene and eugenol derivatives diols with correct stereochemistry were obtained in agreement of the proposed model according our NMR data. For a better understanding, a limonene model is represented in Scheme 9.

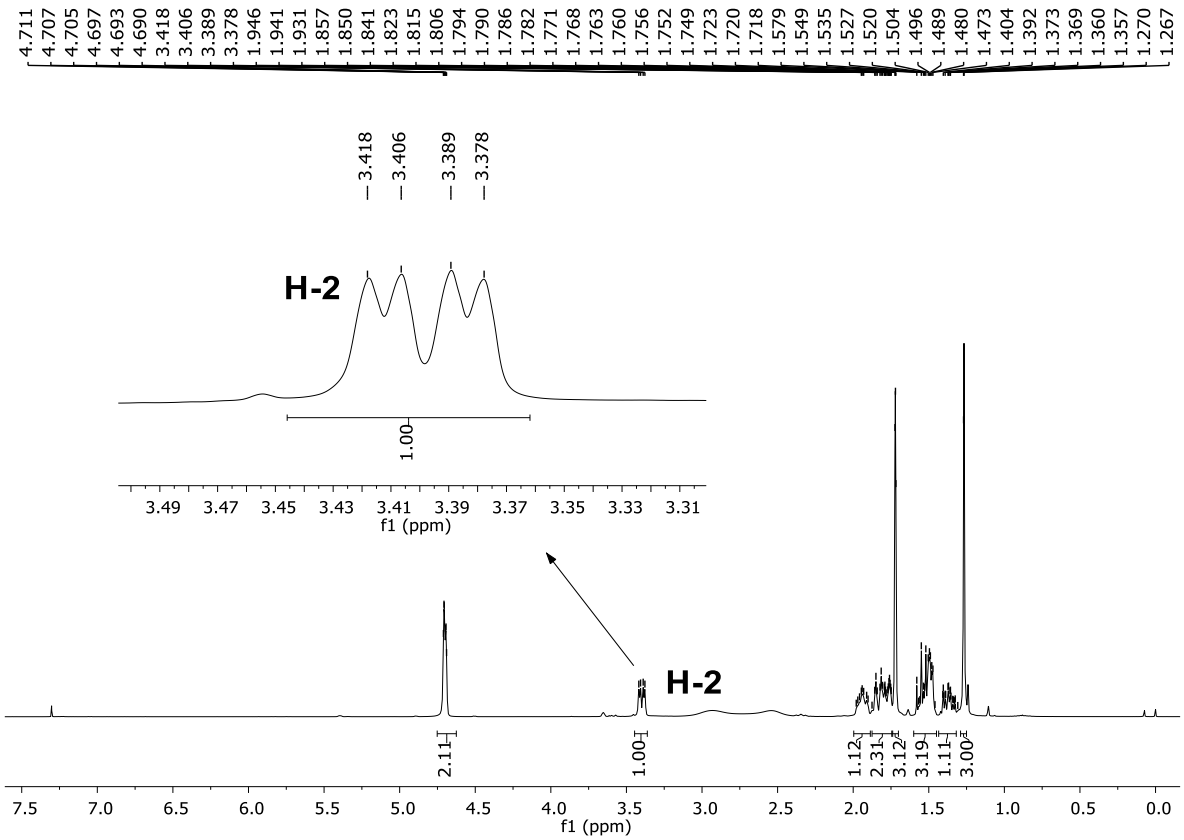


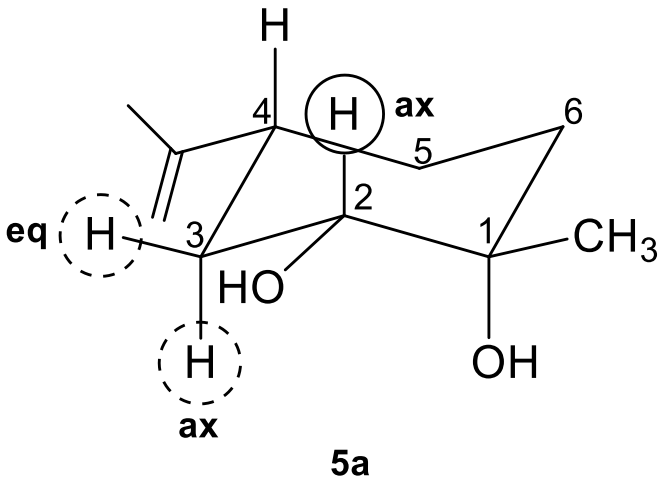
Scheme 9. Addition of the hydroxyl groups to limonene using AD-mix reagents to afford the corresponding enantiomers.

Compound **5a** was identified as a single product with 41% yield. Its conformation shown in Scheme 9 was attributed by the signal of H-2 (dd, δ 3.38) which presents an axial-equatorial (ax-eq) and axial-axial (ax-ax) coupling with the neighbor protons H-3 in ^{1}H NMR (Figure 8). Calculation of the coupling constants *J* agreed with literature (POHVIA, 2014; MORIKAWA *et al.* 2019), as represented in Figure 8.

In the literature, the first report of **5a** was presented in the work of Hao in 2016, but with incorrect stereochemistry and inconsistent data. In 2017, Wang synthesized and described limonene diols using AD-mix-β, but without a detailed mechanism and NMR data. In 2019, Morikawa presented correct NMR attribution and stereochemistry and a rational explanation for the synthesis of asymmetric limonene-1, 2-diols. Our NMR data are in accordance with the findings of Morikawa.

The eugenol diols derivatives were obtained herein for the first time using the Sharpless reaction and the NMR data were attributed for the first time for **7b**. The NMR data of **8a** are in accordance with Sawant and Waghmode (2010).





5a

J H2ax- H3ax = 11.5 Hz
J H2ax-H3eq = 7 Hz

Figure 8. Representation of the configuration of **5a** in agreement with ^{1}H NMR (400 MHz, $CDCl_3$) and *J* calculation for H-2.

The reactional conditions reported herein presents advantages in comparison with the three cited works above. For limonene, Hao *et al.* (2016) used OsO_4, which is very toxic, in contrast with the safer $K_2OsO_4 .2H_2O$ salt used by us. We obtained the limonene derivative in 16 h of reaction without remaining limonene, while Hao et al. in

24 h recovers 23% of the starting material. Wang (2017) used almost the same reaction conditions described by us, but using additional $MeSO_2NH_2$. The 1,2-diol was obtained by Wang in 6 days of reaction with 72% yield, while, we obtained the final product within 16 h and 41% yield. Comparing the reaction time, 6 days is a very long reaction time, and we obtained acceptable yields in only 16 h, making our synthetic efforts more in alignment with the proposed goals of this project. Moreover, further improvement could be made to increase yields.

In the work of Morikawa *et al.* (2019), the limonene-1,2-diols were obtained using as starting materials R-limonene-oxides, that were converted to limonene carbonates in a reactor with 5 MPa under CO_2 pressure and TBA as catalyst. These carbonates were reduced to limonene diols with $LiAlH_4$ in 2 h. In our work, the use of a reactor with pressure was not necessary, and the final products were afforded in a single step, instead of two, with lower reaction times. The cited works are only related to limonene, and we increased the range of reaction by using other substrates as the eugenol ether derivatives, proving the versatility of reaction with interest compounds.

To explore other reaction possibilities, we also tested the AD-mix-α $(DHQ)_2PHAL$ reagent with D-limonene to obtain the other stereoisomer, following the model in Scheme 9. Derivative **5b** was obtained as a single spot with 31% yield, lower than **5a** (41%). It was characterized by NMR data showing the presence of a mixture of diasterisomers through duplicated signals of ^{13}C NMR at δ 149 ppm (Figure 9 and Supplementary Material, Figure S22).

On the other hand, the reaction of eugenol and AD-mix-α reagent afforded a product that could not be identified in NMR and was highly retained in TLC. These results were poor in terms of asymmetric synthesis for limonene and eugenol because using AD-mix-α led to formation of mixtures. Nonetheless, this is the first time that Sharpless reaction using DHQ2-PHAL was reported for limonene and eugenol.

In general, the use of Sharpless reaction to these scaffolds worked with acceptable yields and with a sole stereoisomer formation according the model presented in **Scheme 10**. The conditions were mild with a facile synthesis. The 1,2-diols intermediates (**5a-b** and **8a-b**) obtained in this study were never evaluated before for repellent and insecticide activities.

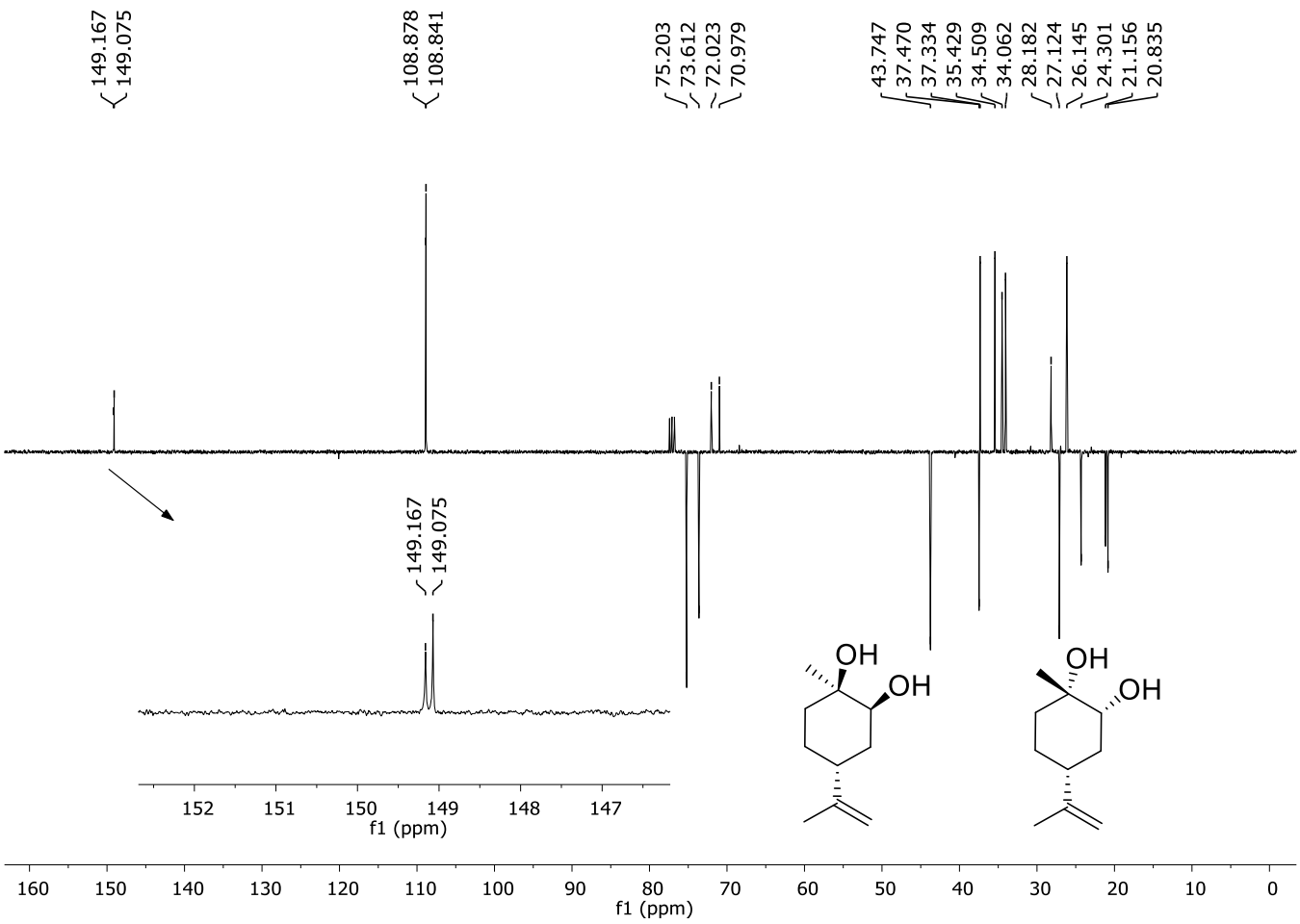
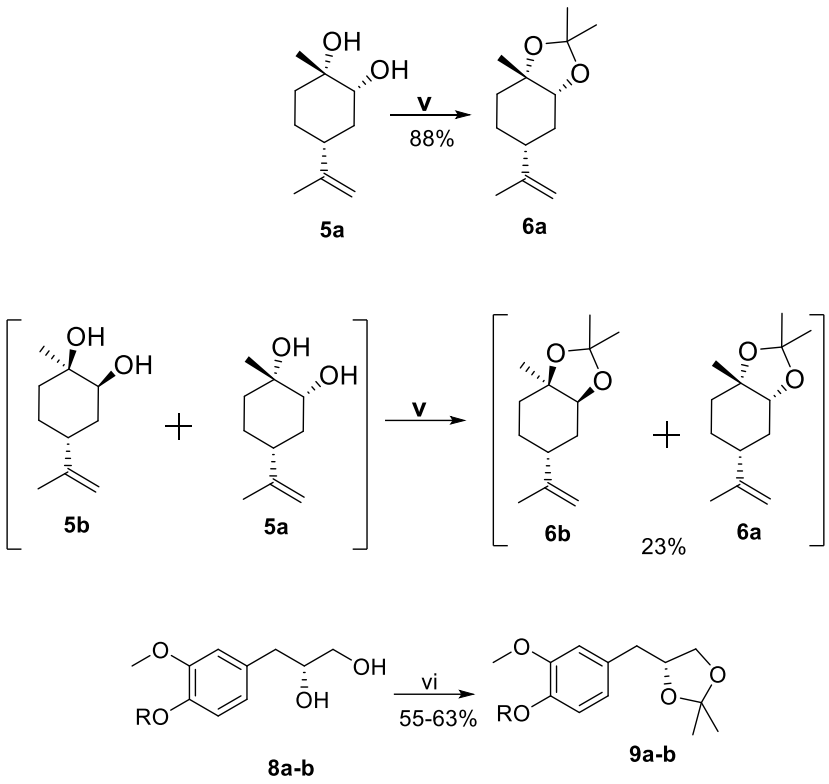


Figure 9. The ^{13}C NMR of compound of mixture **5b/5a**, with a selected zoom showing duplicated signals for all signals, indicating the formatio n of diasterisomers

After the formation of the asymmetric diol, an acetonide reaction with $BF_3.Et_2O$ and dimethoxypropane with the 1,2-diols was accomplished. For **6a,** the yield was 88% and for **6b** (a mixture of diasterisomers) the yield was 23%. For both eugenol products **9a** and **9b**, the yields were 63% and 55%, respectively. **6b** presented a single TLC spot, although it was a mixture. The reaction conditions were based in Andrade *et al.* (2014) but using DMP as a solvent instead of acetone. All of these compounds are new, synthesized by the first time and all the NMR signals attributed. A fully description, including 2D NMR (COSY, HSQC and HMBC) was made for **6a**.



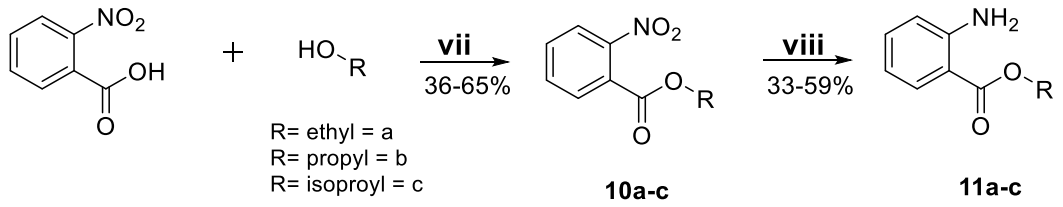
Scheme 10. Acetonide formation using limonene and eugenol diols. Reagents and conditions: 2,2-dimethoxypropane, $BF_3.Et_2O$, 8 h; Compound **6b** is obtained as a mixture with **6a**.

The formation of final products was verified by the acetonide shift at ^{13}C δ 108-110 ppm and HRMS data for **6a, 9a** and **9b**. These are new compounds so not only repellency, but also other activities could be explored for them.

3.4 Esterification with EDC/DMAP and reduction of the NO_2 group

Starting by 2-nitrobenzoic acid, esterification was the first step using EDC as a coupling agent and DMAP as the catalyst with the desired alcohols in CH_2Cl_2. The methodology is based in Steglich esterification (NEISES and STEGLICH, 1978) using EDC instead of DCC. The reactions occurred with no problems at room temperature overnight with a single TLC spot for each one. The three esters **10a**, **10b** and **10c** were obtained with yields of 65%, 60% and 36%, respectively. All NMR data were attributed

(Supplementary Material). The formation of ester derivatives were identified by the corresponding ^{13}C NMR signals around at δ 165.5 (POHVIA, 2014). HRMS data agreed with the proposed molecules.



Scheme 11. Preparation of nitrobenzoates and anthranilate esters. Reagents and conditions: **vii**- EDC, DMAP, appropriate alcohol, 25 ºC, 16 h; **viii** – SnCl2, EtOAc, 60 ºC, 4 h.

Then, all the nitrobenzoate compounds (**10a-c**) were submitted to $SnCl_2$ for reduction of the NO_2 group in distillated ethyl acetate for 4 h, adapted from Bellamy and Ou (1984). The mixture was filtered via a celite plug with the obtention of the pure anthranilic esters. The products **11a**, **11b** and **11c** presented the same trending yields as the nitro products with 59%, 40% and 33% yields. The formation of NH_2 was first identified by TLC with ninhydrin and confirmed by NMR data. Presence of a new singlet at δ 5.6-5.7 was attributed to NH_2 group. HRMS data agreed with the proposed molecules.

The compounds, obtained from the esterification and the reduction reactions, were purified by filtration and with acceptable yields. The variation of yields were due to increase of volume of the alcohol. Smaller ethanol presented better yields than voluminous isopropanol and propanol. In general, reactions were fast, mild and reliable.

3.5 Biological activity – preliminary results

The tests are based in the module designed and presented in the Materials and Methods sections. All tests were realized in controlled environment and the data were treated before presentation. In the present work, molecules were tested for repellency, including starting materials and intermediates. As the starting materials are natural repellents, comparison in this section was made with their synthetic counterparts as well as with the positive control (DEET). So, D-camphor and L-menthone were compared with their acetals and lactones. Limonene and eugenol were compared with diols and acetonides. In the case of eugenol, their ether derivatives were also compared. For the anthranilate series, the comparison was made with the nitro benzoate esters. The negative control is EtOH.

The repellent activity was determined by the number of landings (Fig 8 and Fig. 9), feeding (Fig 10) through membranes impregnated with tested compounds and the Sting Protection Index (P). The concentration was settled for 200 nmoles/cm^2 for all compounds.

Initially, the starting materials (eugenol, D-limonene, L-menthone and D-camphor) did not present any significant difference with negative control in the tested concentration for both landing and feeding tests, and in the P value. In intervals of 1 min or along 5 min, the number of landings is higher than EtOH (>4), as well for ingurgitate mosquitoes.

The anthranilate esters, known as repellents, presented limited activity, with no significant difference than negative control. Ethyl anthranilate **11a** did not present significant activity in the tested concentration in comparison with DEET. On the opposite of this result, **11a** attracted attention in literature recently because it had promising repellent activity in a 600 mM concentration (ISLAM, 2017; ZHAO *et al.*, 2019). The nitrobenzoate esters **10a** and **10c** presented repellent activities comparable to DEET, and with P values of 0.70 and 0.69. (DEET, P value = 0.86)

Considering the intermediate compounds, limonene diols (**5a** and **5b**) presented nearly three times lesser numbers of landing and ingurgitated mosquitoes (≈1) than limonene (≈3 for landing and >4 for ingurgitated). **5a** and **5b** presented repellent activities comparable to DEET and good values of P, with 0.71 and 0.62 respectively.

In the case of eugenol, the ether **7a** is one of the most active compounds (>1 but <2, for landing and ingurgitated, P value of 0.69), suggesting that the methylether group greatly increased the repellency in comparison with phenol group. However, for **8a,** the presence of the terminal asymmetric diol abolished the activity (≈3), with no difference to the negative control and a poor P value (0.33). Conversion of **8a** to acetal **9a** restored the activity, being the acetal similar in numbers of landing, feeding and P values, than **7a**. In conclusion, the conversion of eugenol to ethers presented similar outcomes as with their acetals. **7a** and **9a** presented repellent activities comparable to DEET.

For menthone, the lactone **4** is almost three times (≈2) more active in the number of ingurgitated mosquitoes than the starting material (≈6), while for number of landings (≈2), **4** is at least one degree lower than menthone (>3 but <4). The P value is 0.62, next to the most active compounds in this study. Lactone **4** presented repellent activities comparable to DEET.

In summary, the nitroester **10a** was the most active derivative for repellency, followed by limonene diols (**5a**, **5b**), eugenol ether (**7a**) and acetal (**9a**), lactone of menthone (**4**) and aminobenzoate ester (**10c**). The most active compounds of the series were **10a**, **9a** and **5a** considering the number of landings, feeding and P value.

Compound **10a,** named 2-nitroethylbenzoate, was already synthesized (MACDONALD *et al.*, 2018), but it was never studied for repellent activity so far. However, 2-nitromethyl benzoate, a compound similar to **10a**, in a recent study against gypsy moth larvae and brown marmorated stinkbug nymphs, presented no larvicidal, ovicidal, nymphicidal and insecticidal activity (FENG *et al.*, 2018). This could be a good indication of the potential of **10a** as a repellent. If the same is not toxic against larvae, then it is very similar to DEET, the gold standard. DEET presents only repellence, and not pesticide activities, being ideal to deter or repel arthropods. As the activity for **10a** is comparable to DEET in our data in all tested scenarios, more experiments are necessary before it could be a potential commercial repellent.

The limonene diol **5a** was already synthesized (WANG *et al.*, 2017; MORIKAWA *et al.*, 2019), but it was never studied for repellent activity so far. In our tests, **5a** presented similar results to DEET. Yet, this asymmetric diol presented good repellent activity, and is a promising compound.

Lastly, **9a** is a novel asymmetric acetal and to date it was never described in the literature. Its repellent activity was similar to **7a, 5a**, and DEET. It was demonstrated that modifying eugenol to ethers or acetals could increase repellency. All these compounds (**5a, 9a, 10a**) have potential to be developed to commercial repellents.

Taking all of these results in consideration, the most active compounds in general, in this study were **5a**, **7a**, **9a**, **10a** e **10c**.

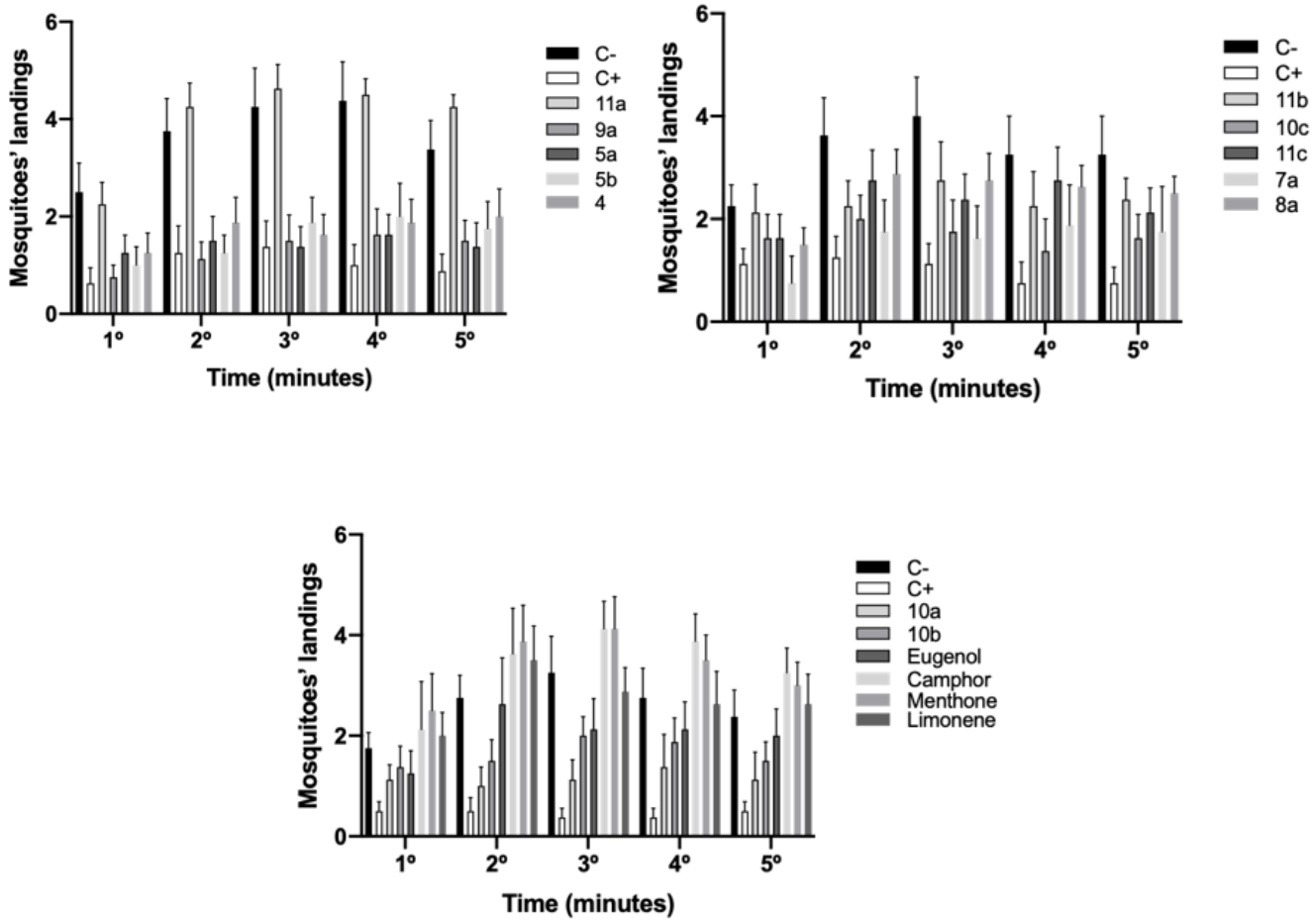


Figure 10. Average number of landing, of female adult *Aedes Aegpty* in membranes impregnated with tested compounds (200 nmoles/cm^2) in intervals of 1 min. Anova on ranks (Kruskal-Walis) and comparations by Dunn test. Different letters indicate significant differences between the treatments and the negative control (P<0,05). C- = negative control (EtOH); C+ = DEET. N=40

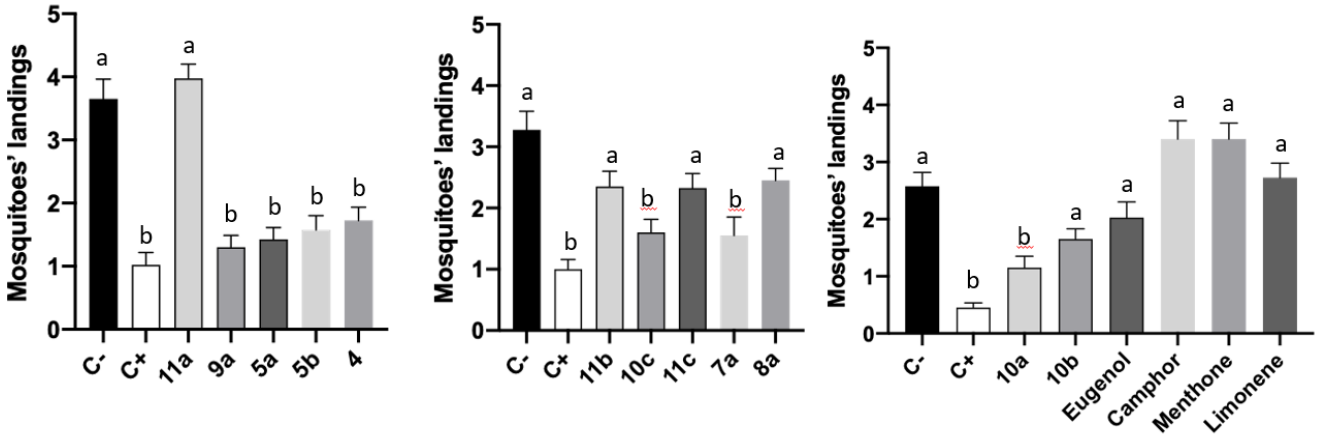


Figure 11. Average number of landing, of female adult *Aedes aegpty* in membranes impregnated with tested compounds (200 nmoles/cm^2) along 5 minutes. Anova on ranks (Kruskal-Walis) and comparations by Dunn test. Different letters indicate significant differences between the treatments and the negative control (P<0,05). C- = negative control (EtOH); C+ = DEET. N=40

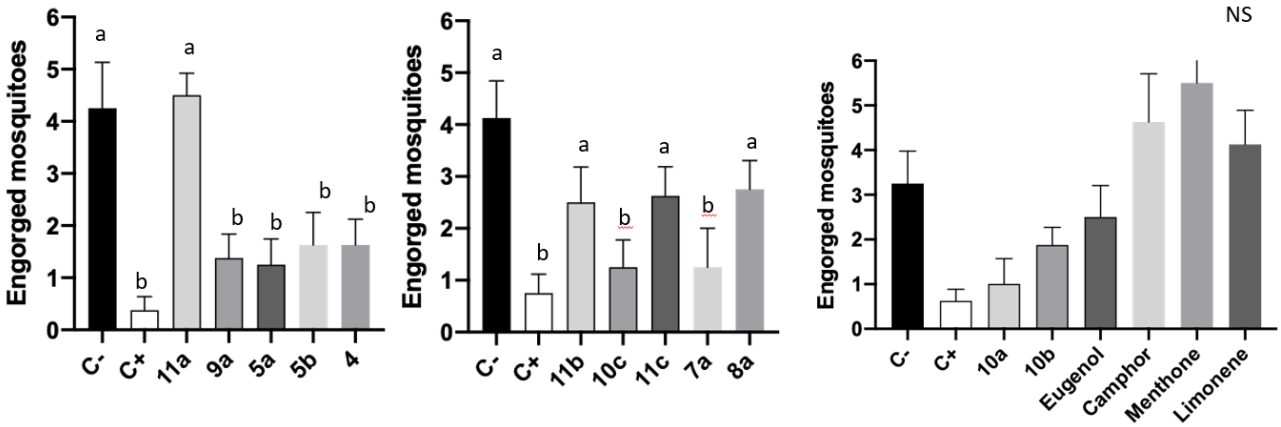


Figure 12. Average number of female adult *Aedes aegpty* that feeded through the membranes impregnated with tested compounds (200 nmoles/cm^2) along 5 min. Anova on ranks (Kruskal-Walis) and comparations by Tukey test. Different letters indicate significant differences between the treatments and the negative control (P<0,05). C- = negative control (EtOH); C+ = DEET. N=40

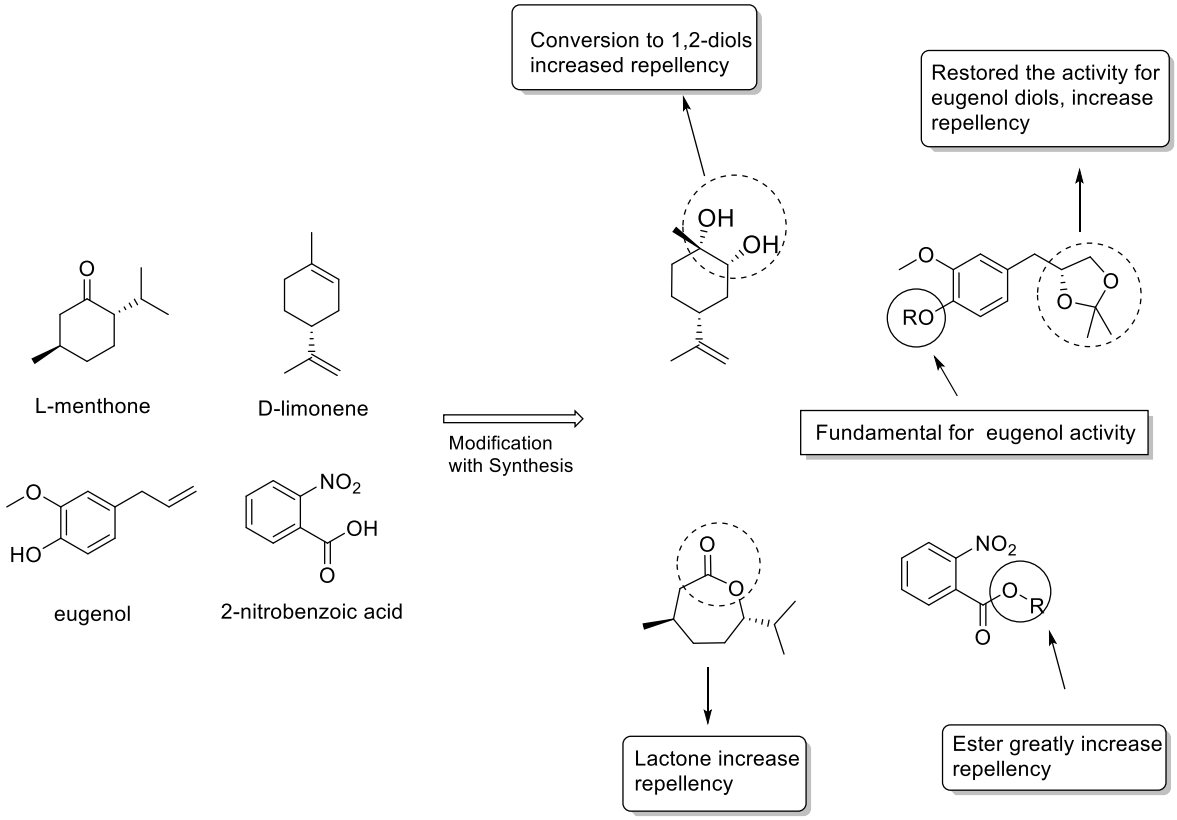
Table 1. Sting Protection Index (P) value of various compounds tested at (200 nmoles/ cm^2) against *Aedes aegypti* aggression, in intervals of 5 minutes. NC = no calculated (Number of images equal to or larger than negative control)

Compound	P
DEET	0.86
11a	NC
9a	0.68
5a	0.71
5b	0.62
4	0.62
11b	0.39
10c	0.69
11c	0.36
7a	0.69
8a	0.33
10a	0.70
10b	0.42
Eugenol	0.23
Camphor	NC
Menthone	NC
Limonene	NC

Finally, it was possible to propose some structural relationships regarding the repellent activity and the tested compounds so far.

3.6 Structure Activity Relationships

For the tested compounds, the introduction of specific functional groups increased repellency in counterpart with the starting materials. The main observations are represented in Scheme 12.



Scheme 12. Structure-Activity Relationships for the most active compounds as repellents to *Aedes aegypti*.

In the first batch of testing compounds, the introduction of assymetric 1,2-diols in D-Limonene via SAD, was beneficial for activity for both compounds **5a** and **5b**. Even **5b** being a mixture it presented relevant results. However, **5a** is more active than **5b,** indicating that a specific isomer is more active than the mixture, proving that selectivity can increase repellency. Unfornately, the 1,2-diol in alkylated eugenol derivatives was not very active in comparison with limonene series and abolished activity at 200 nmoles/cm^2 concentration.

Alkylation in eugenol derivatives in comparison with eugenol itself and the 1,2 diols, greatly increase the repellency and is proven as a fundamental part of the structure for activity.

Conversion of the menthone and 2-nitrobenzoic acid to esters improved dramatically the activity, where the most active compound come from nitrobenzoate series. In this case, the presence of the p-nitro group are necessary. When converting to nitro to amines, the molecules are not effective at the tested concentration. Other ester, lactone also present a significant repellency. With exception of anthranilic series, the presence of esters are essential for the observed repellency.

4. CONCLUSION

In conclusion, 23 molecules were synthesized and evaluated the repellency in *Aedes aegypti*. Five compounds presented repellent activity similar to DEET. The most active series, nitrobenzoates have the ester group and a nitro in the para position. We are still waiting the results for the series of camphor and the limonene acetals in order to help us understand the effects of this functional group in repellency. With the given results, we can propose the continuation of the studies to clarify the relationships between structure and activity and to investigate the potential and safety of the most promising derivatives.

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6.SUPPLEMENTARY MATERIAL

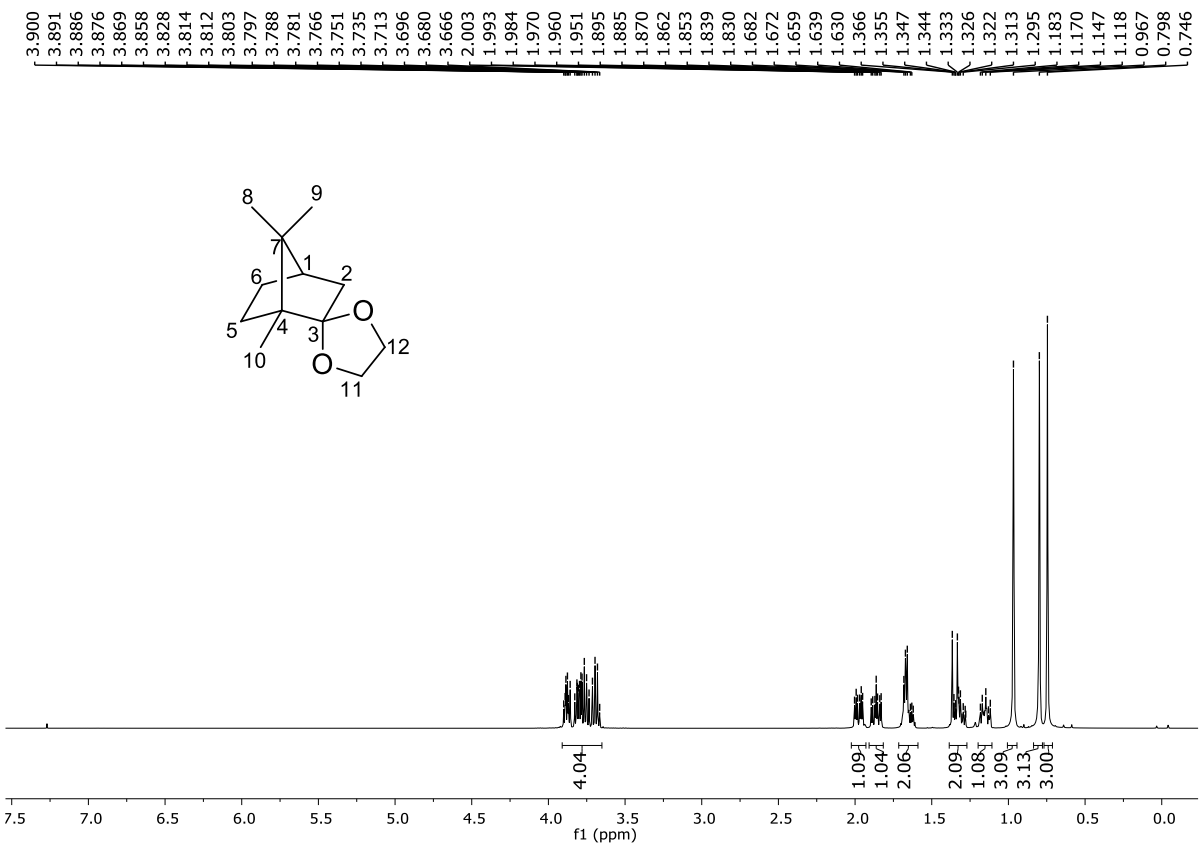


Figura S1. ^{1}H RMN for compound **1a** ($CDCl_3$, 400 MHz)

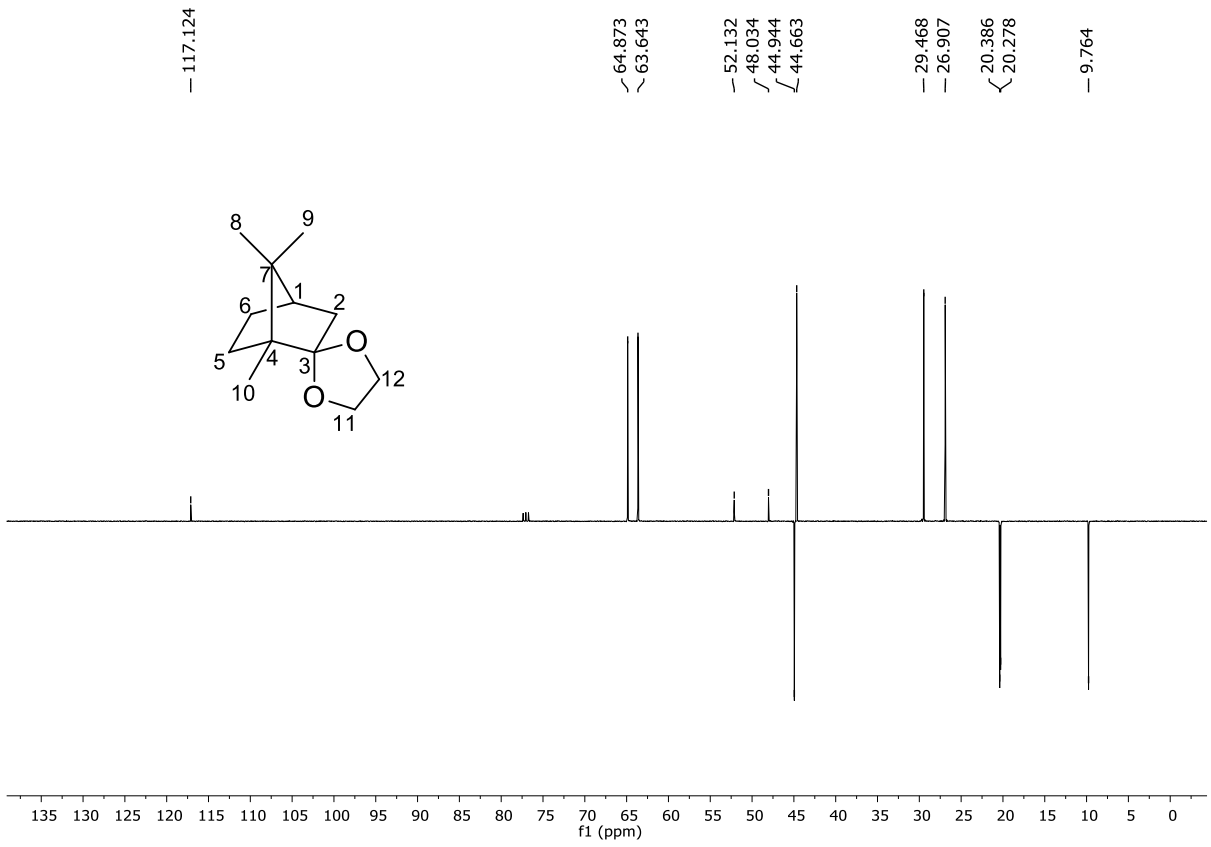


Figura S2. ^{13}C RMN APT for compound 1a ($CDCl_3$, 101 MHz)

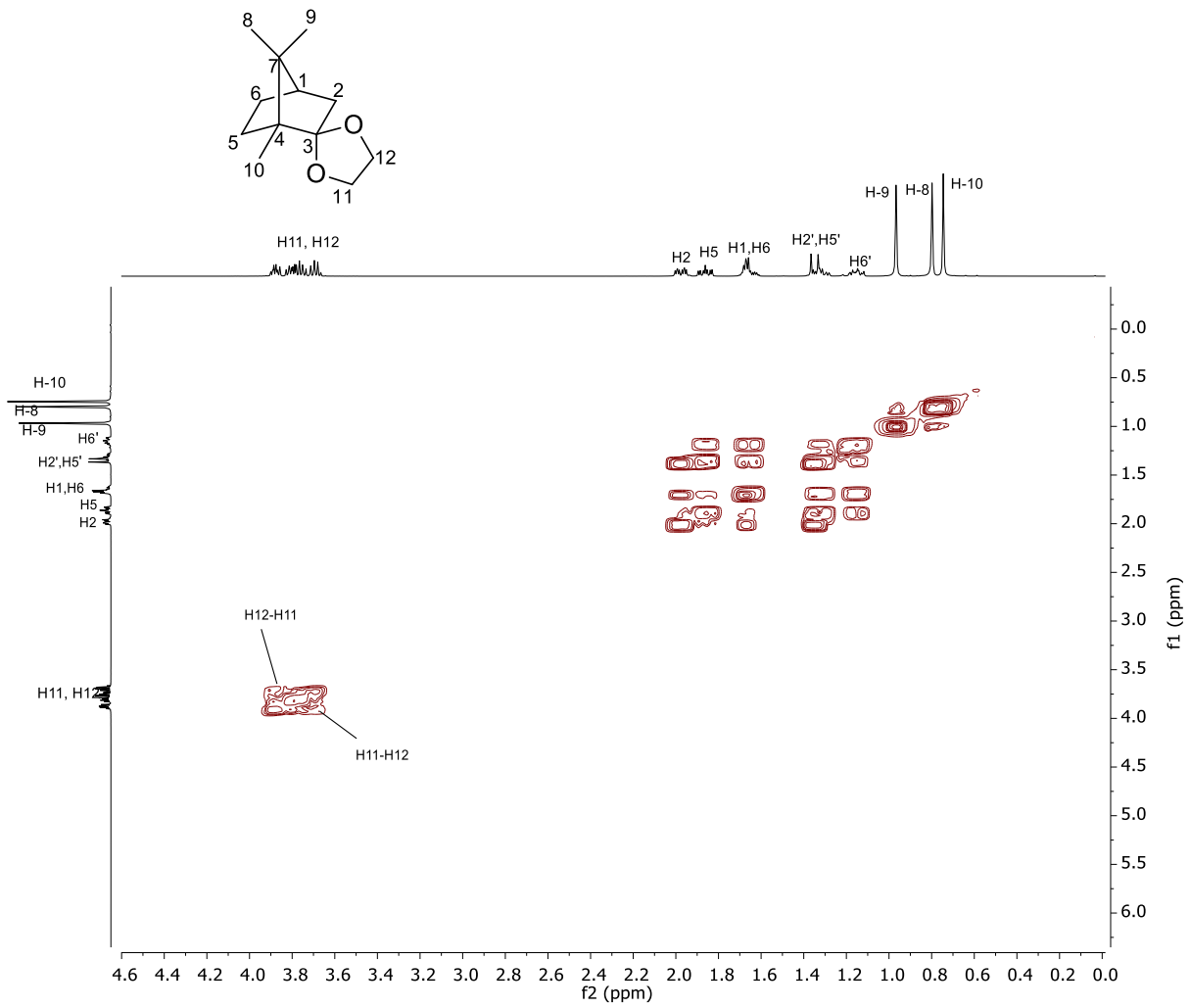


Figure S3. ^{1}H-^{1}H COSY for compound 1a, full spectra with high sensibility ($CDCl_3$, 400 MHz)

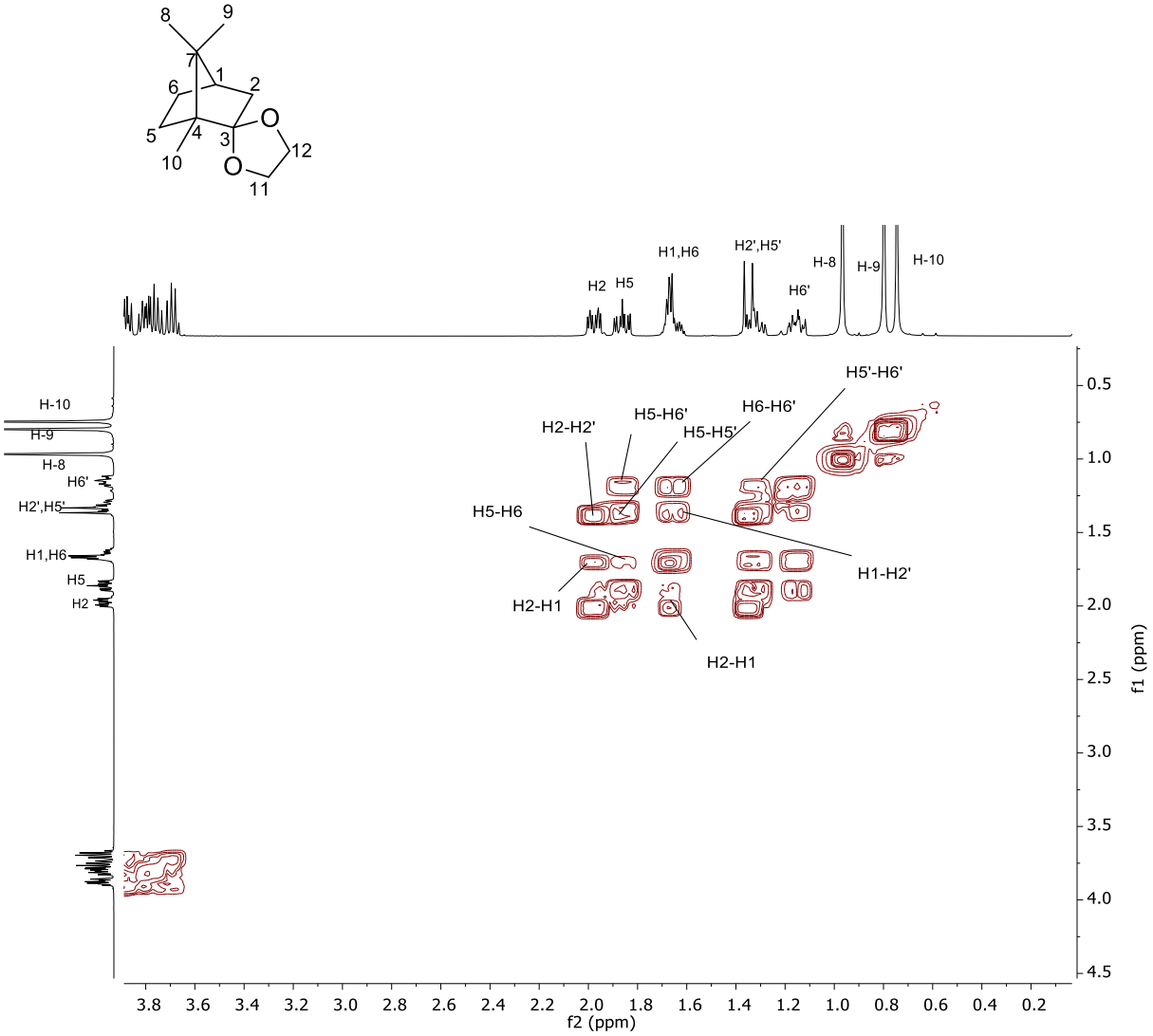


Figure S4. ^{1}H-^{1}H COSY for compound 1a, selected range with high sensibility ($CDCl_3$, 400 MHz)

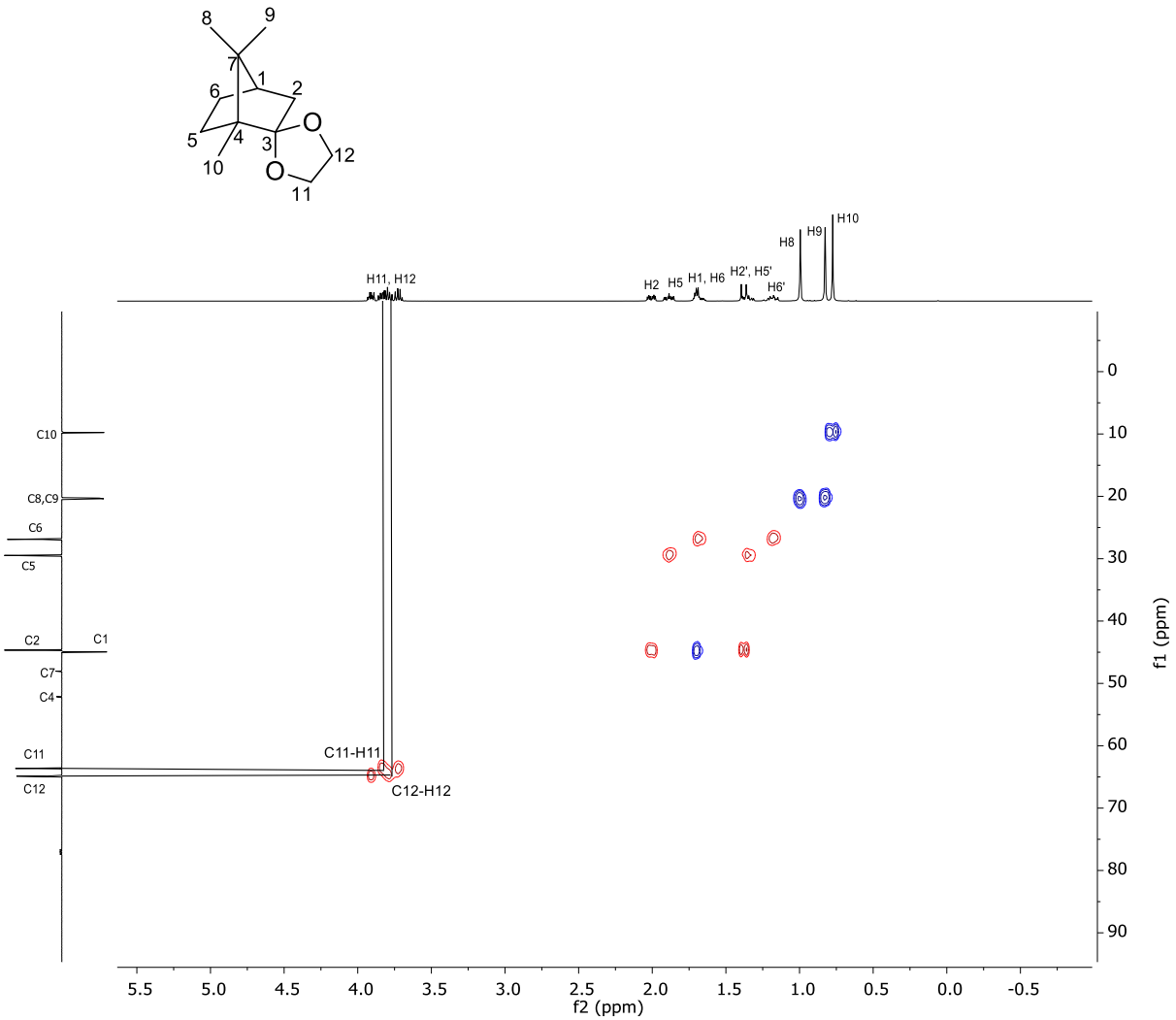


Figure S5. ^{1}H-^{13}C-HSQC for compound 1a, full spectra with high sensibility ($CDCl_3$, 400 MHz)

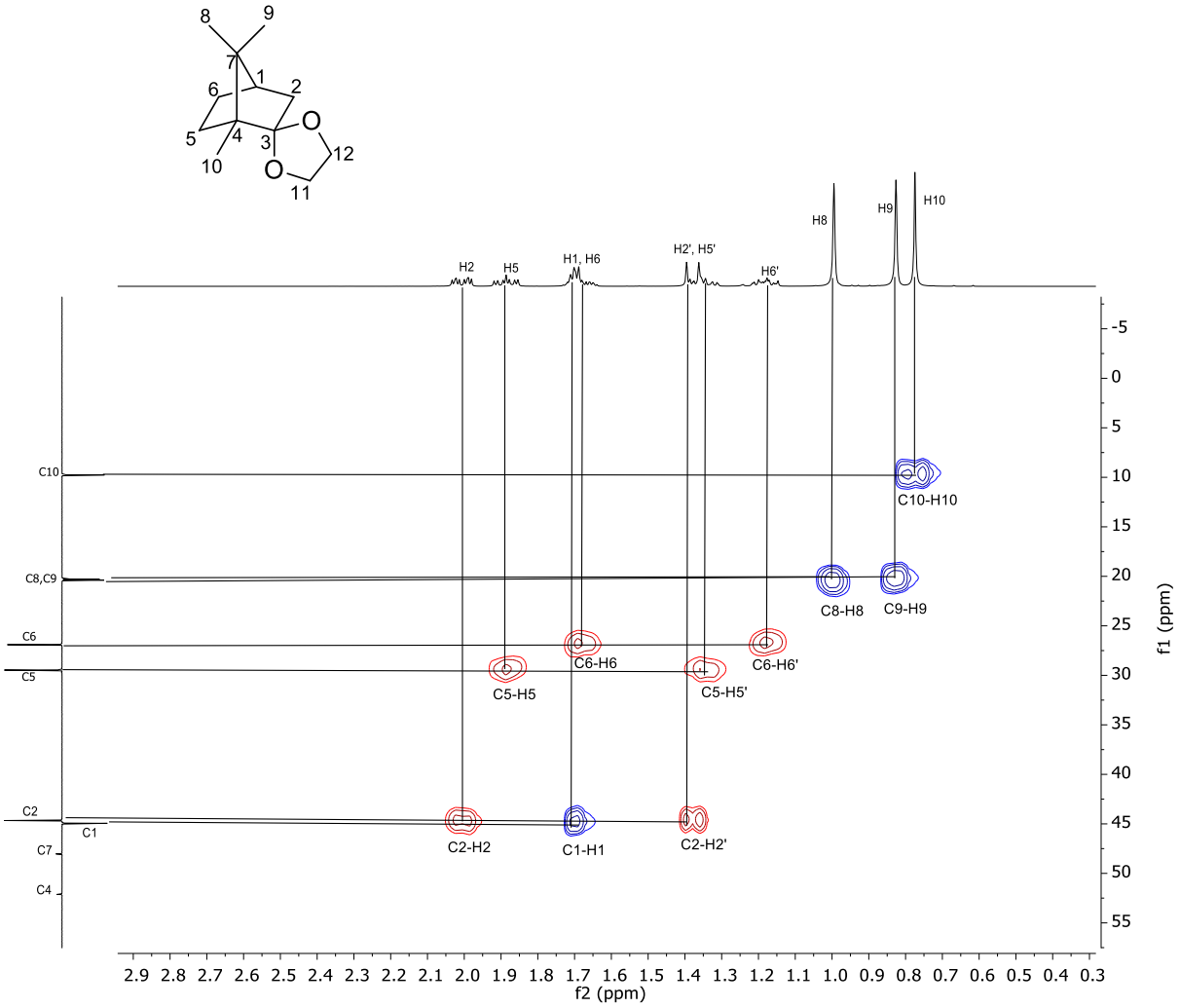


Figure S6. ^{1}H-^{13}C-HSQC for compound 1a, selected range with high sensibility ($CDCl_3$, 400 MHz)

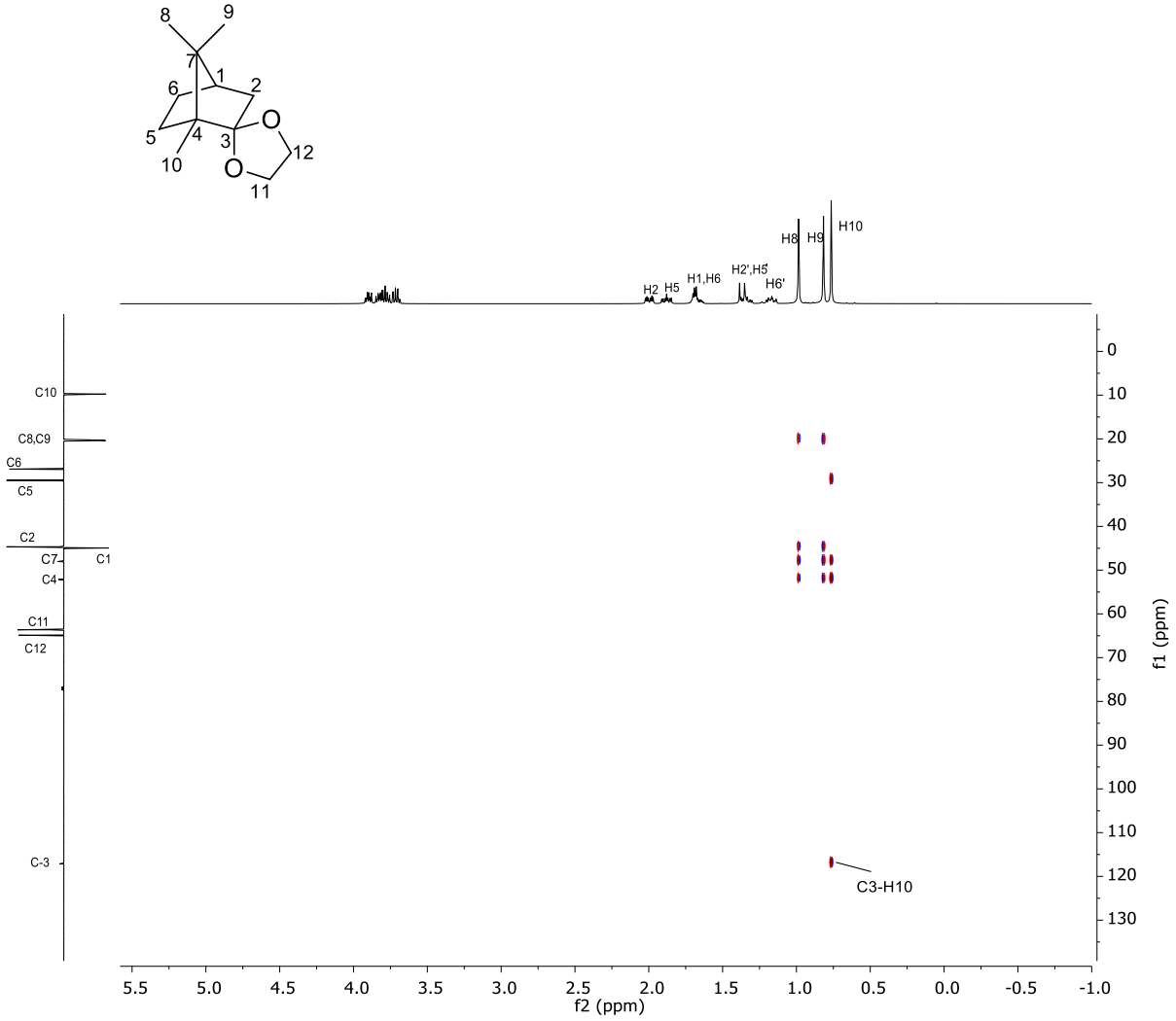


Figure S7. ^{1}H-^{13}C-HMBC for compound 1a, full spectra with high sensibility ($CDCl_3$, 400 MHz)

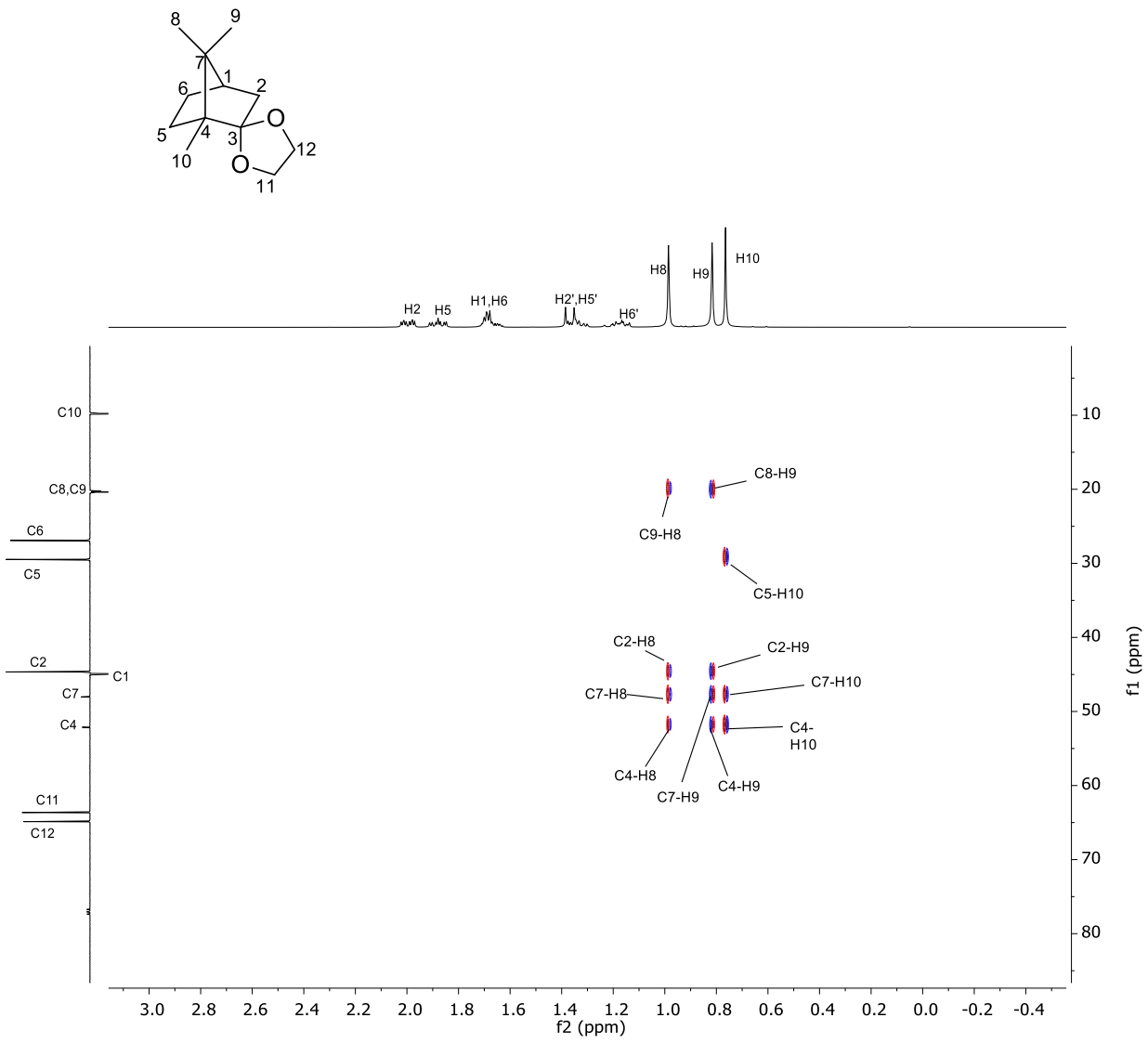


Figure S8. ^{1}H-^{13}C-HMBC for compound 1b, full spectra with high sensibility ($CDCl_3$, 400 MHz)

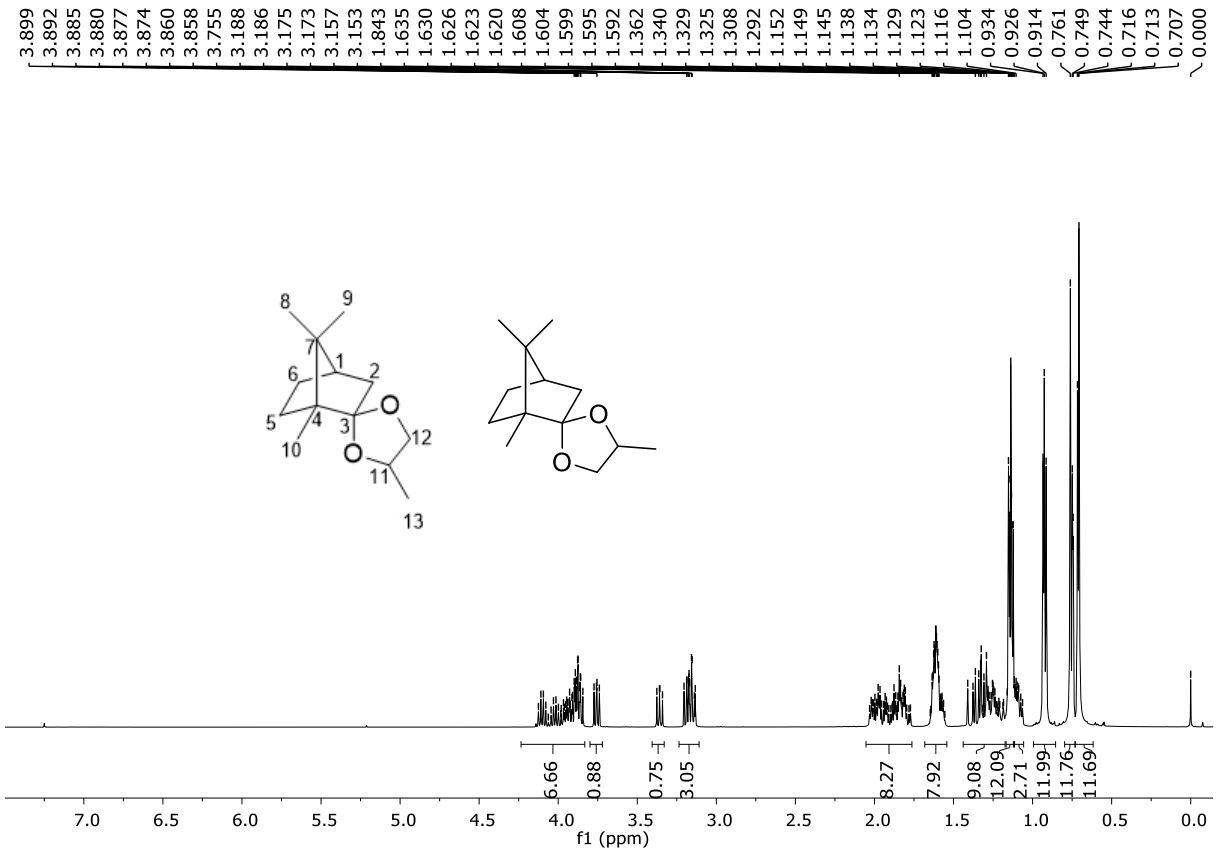


Figure S9. ^{1}H NMR for mixture 1b/1c (mixture of diasterisomers). ($CDCl_3$, 400 MHz)

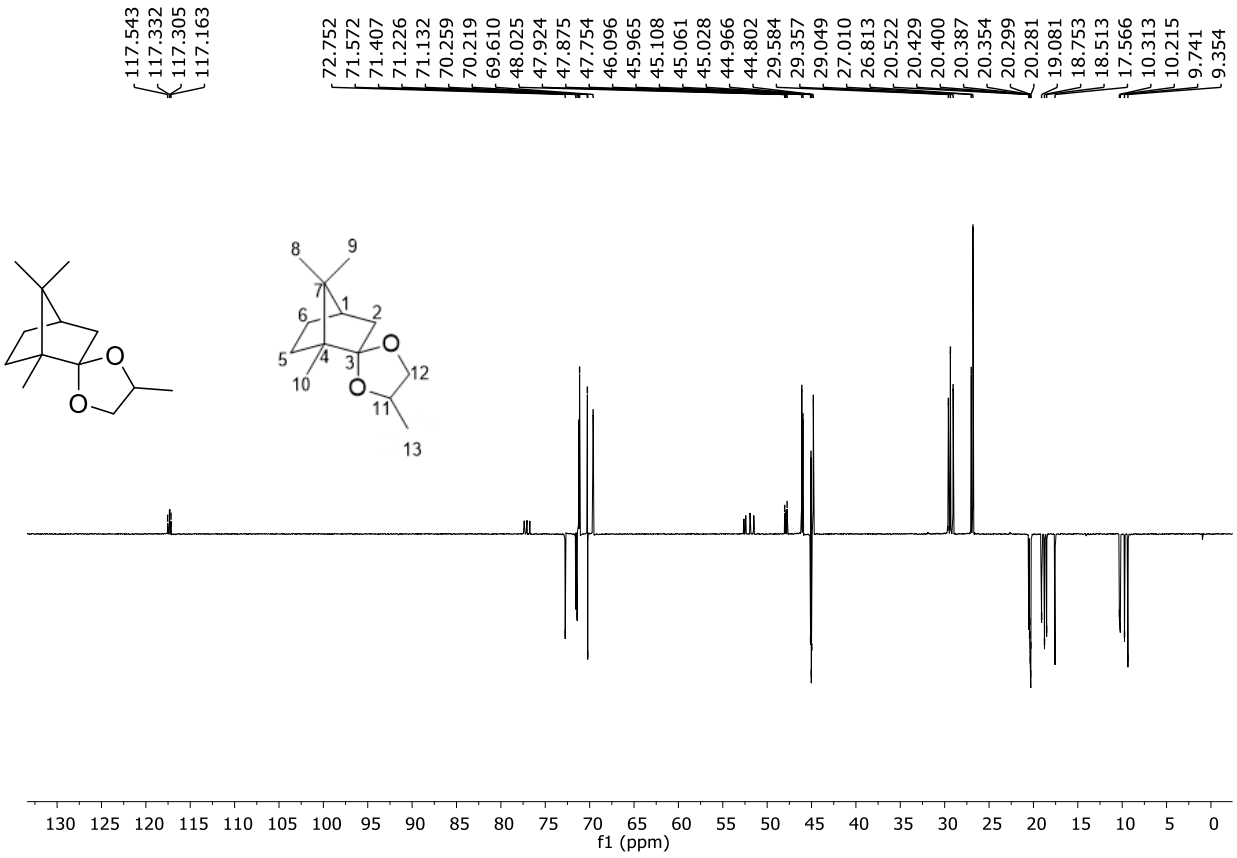


Figure S10. ^{13}C APT NMR for mixture 1b (mixture of diasterisomers). ($CDCl_3$, 101 MHz)

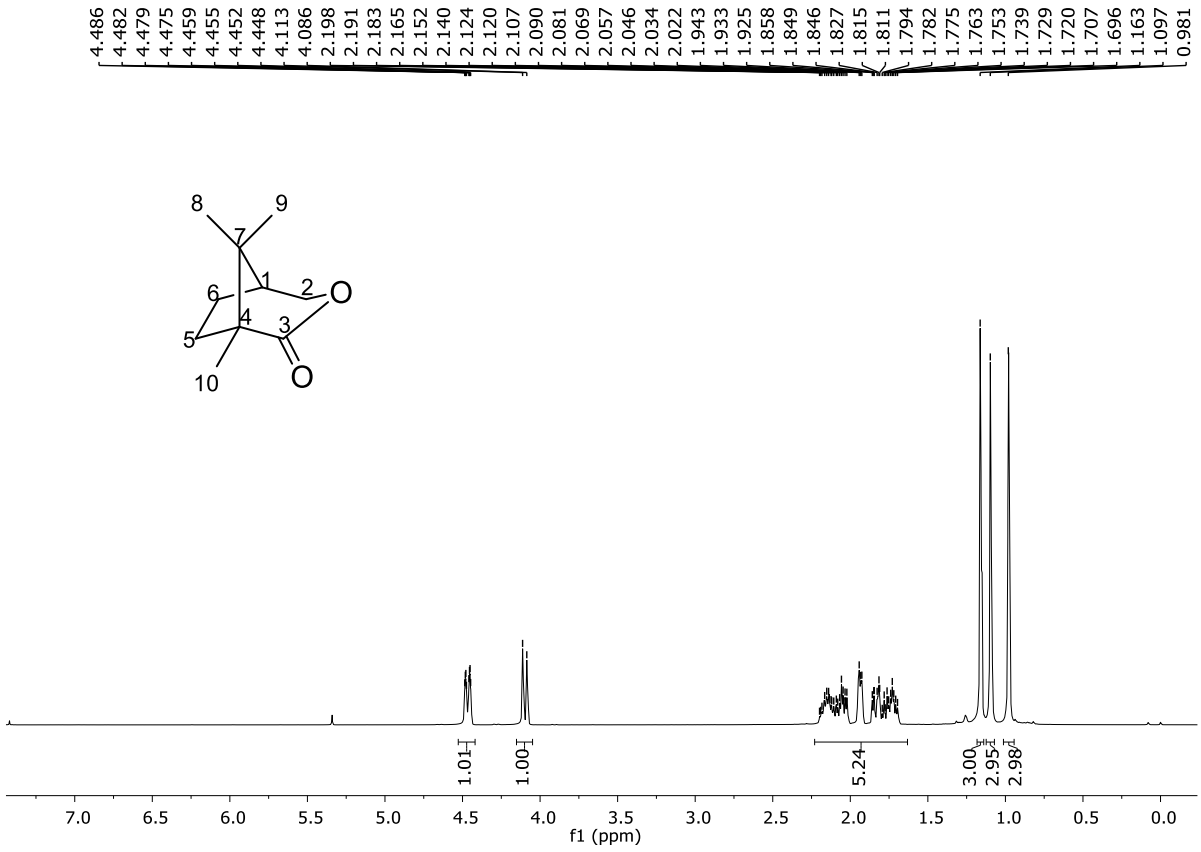


Figure S11. ^{1}H NMR for compound 2. ($CDCl_3$, 400 MHz)

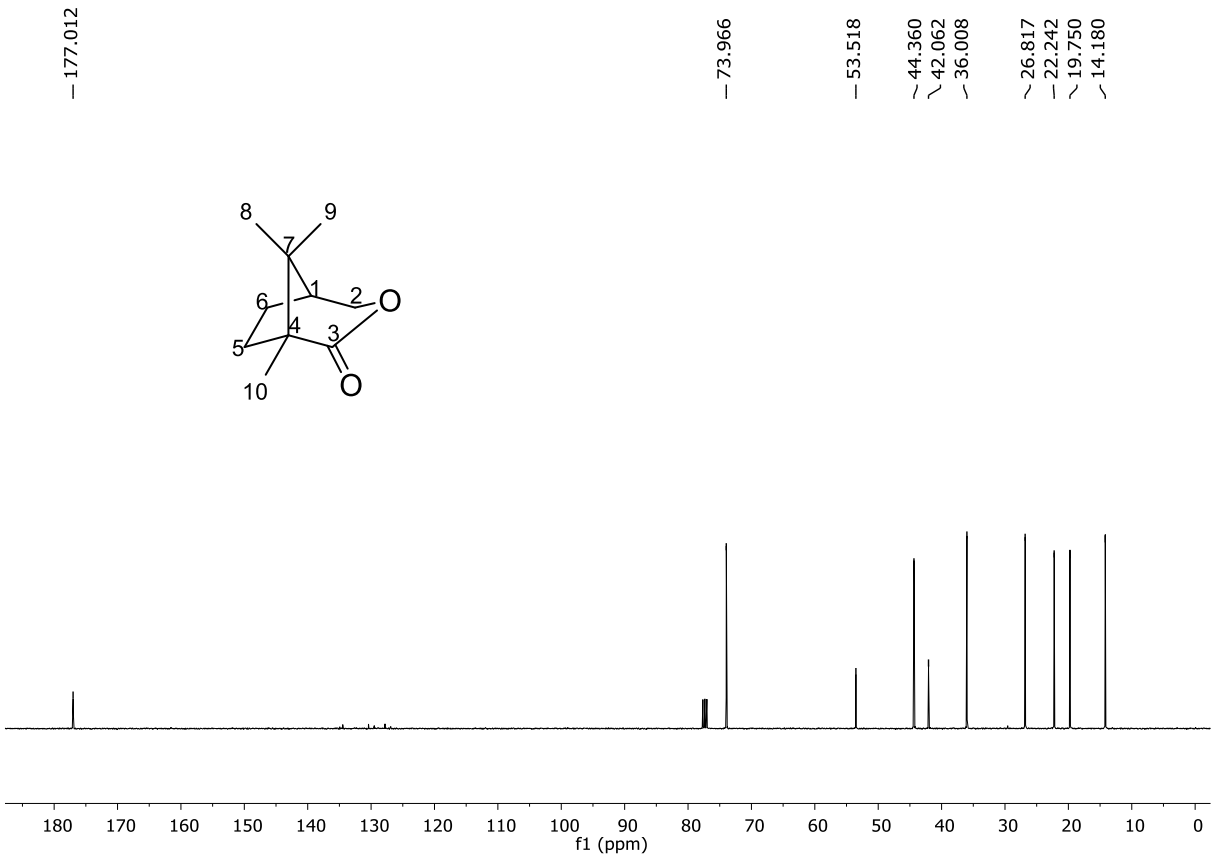


Figure S12. ^{13}C NMR for compound 2. ($CDCl_3$, 101 MHz)

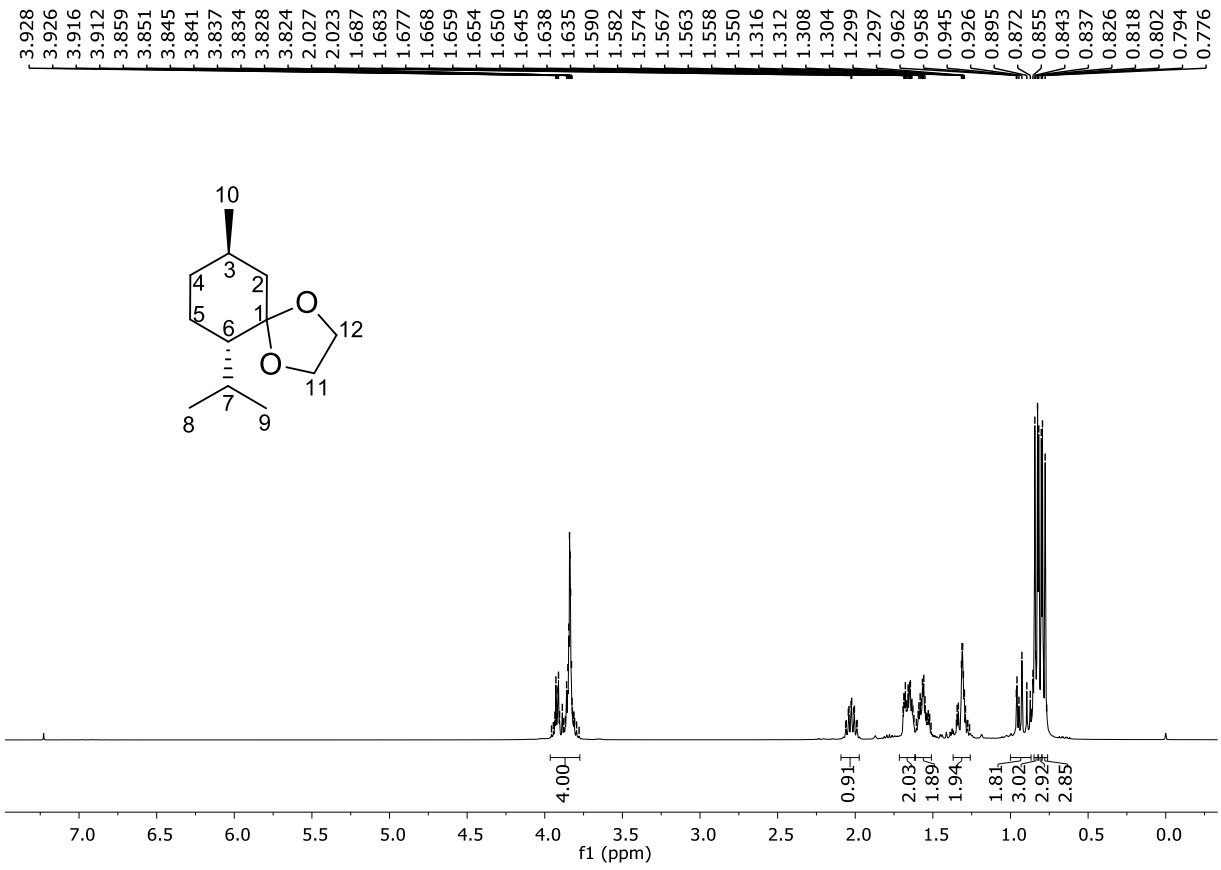


Figure S13. ^{1}H NMR for compound 3a ($CDCl_3$, 400 MHz)

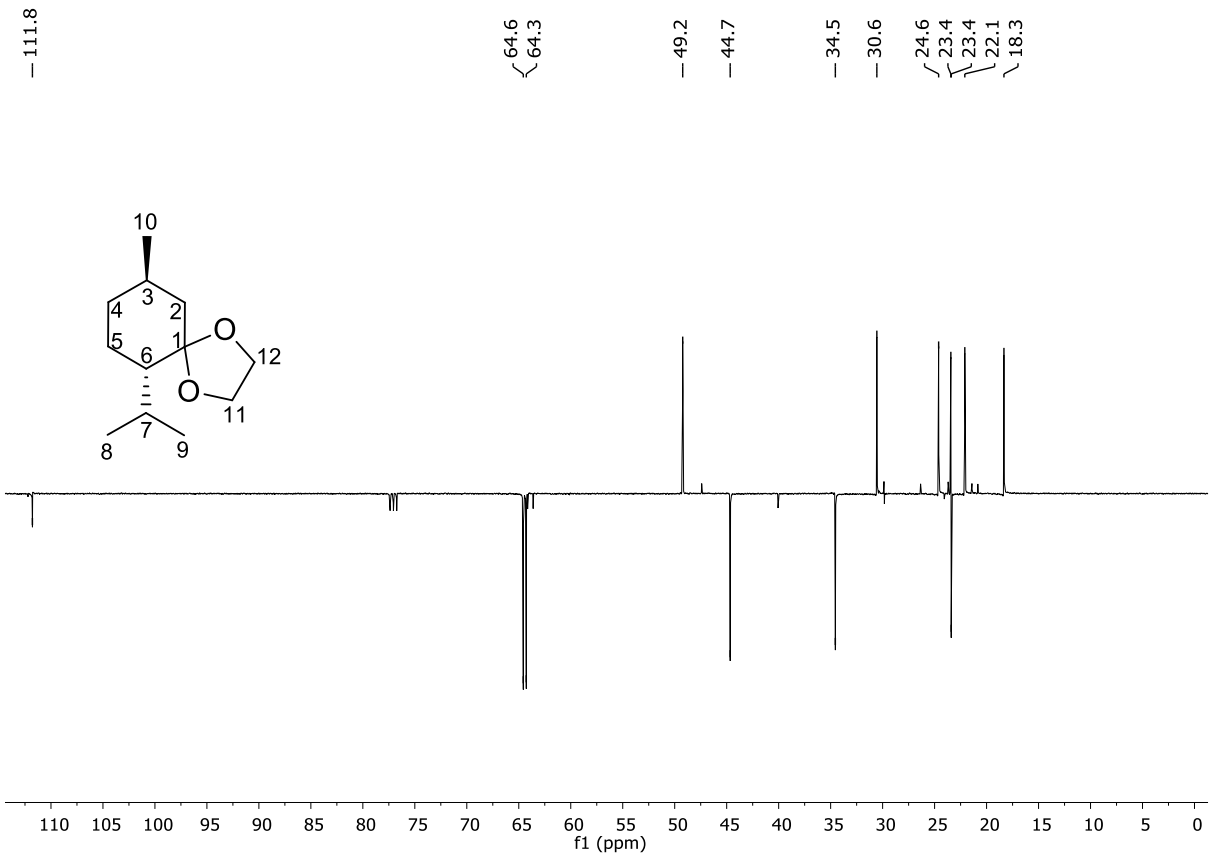


Figure S14. ^{13}C NMR APT for compound CH17. ($CDCl_3$, 101 MHz)

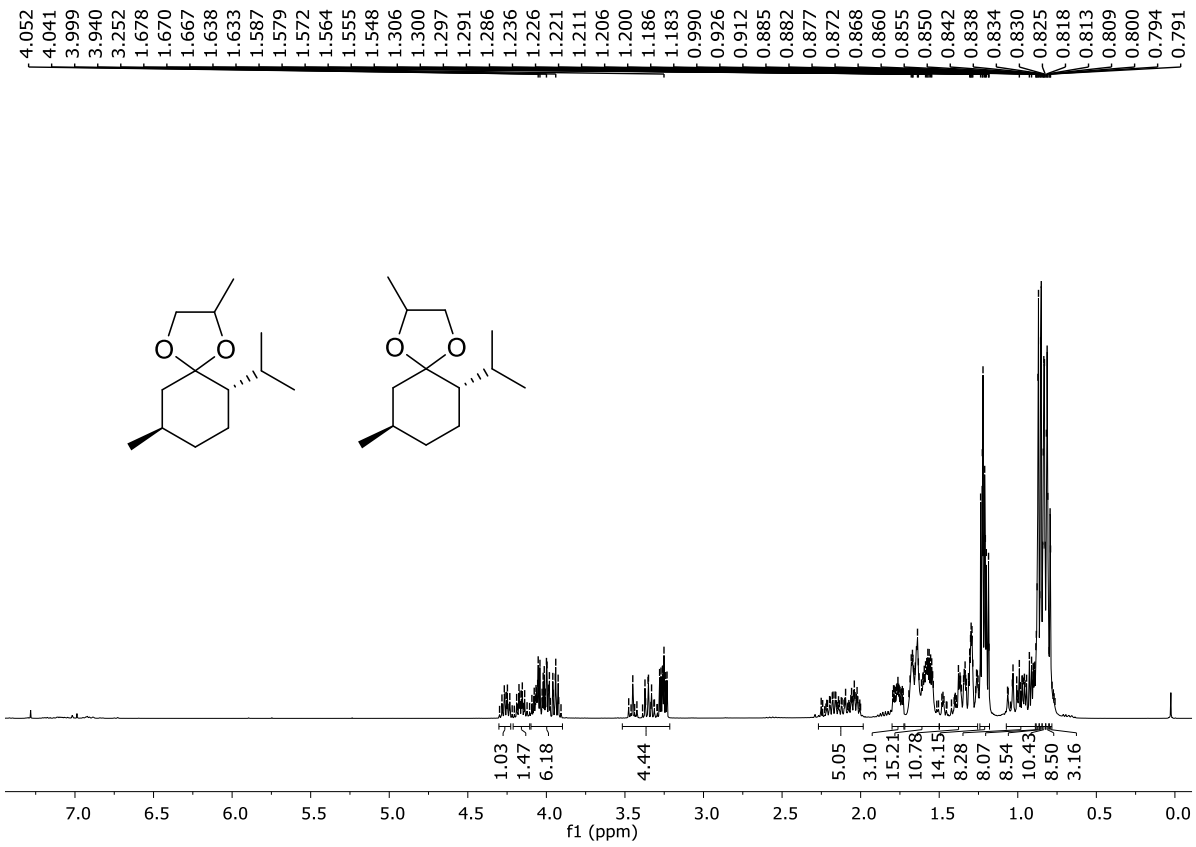


Figure S15. ^{1}H NMR for mixture 3b/3c ($CDCl_3$, 400 MHz)

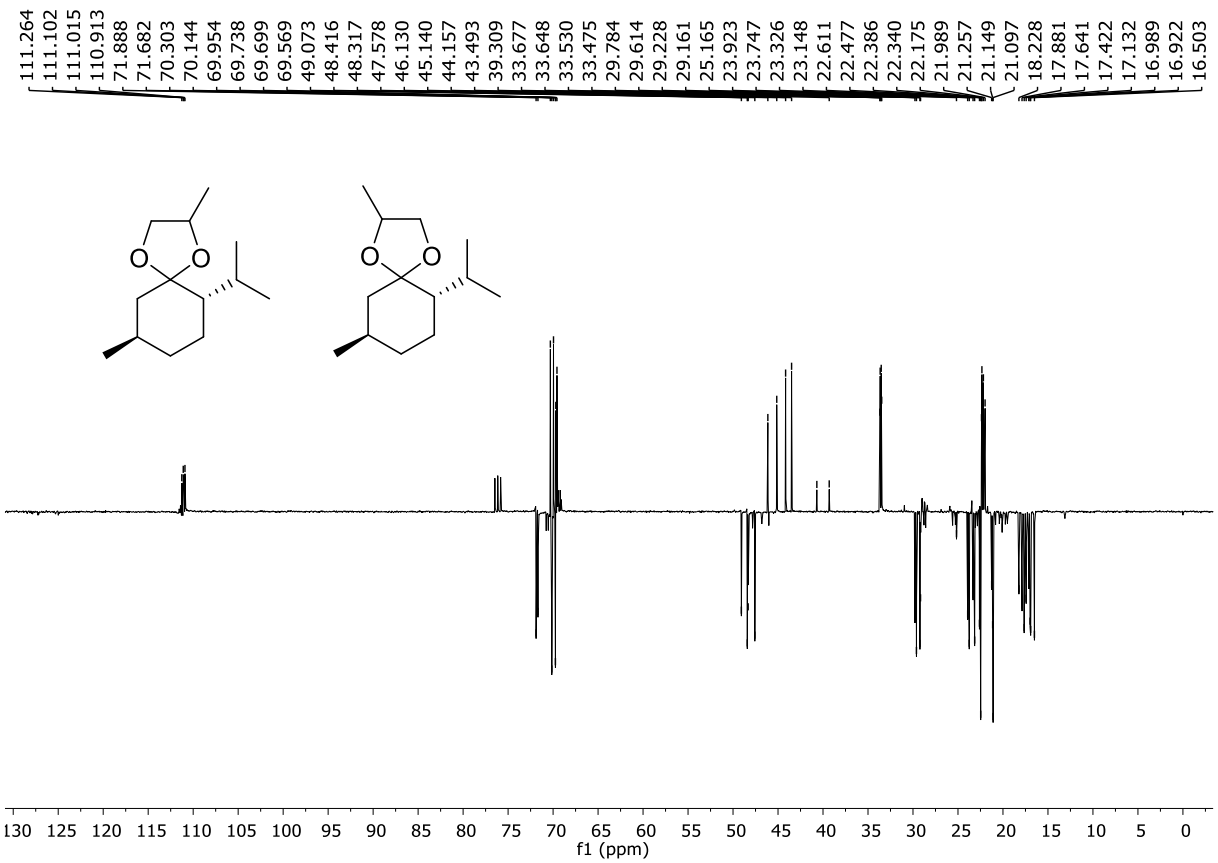


Figure S16. ^{13}C NMR for mixture 3b/3c ($CDCl_3$, 101 MHz)

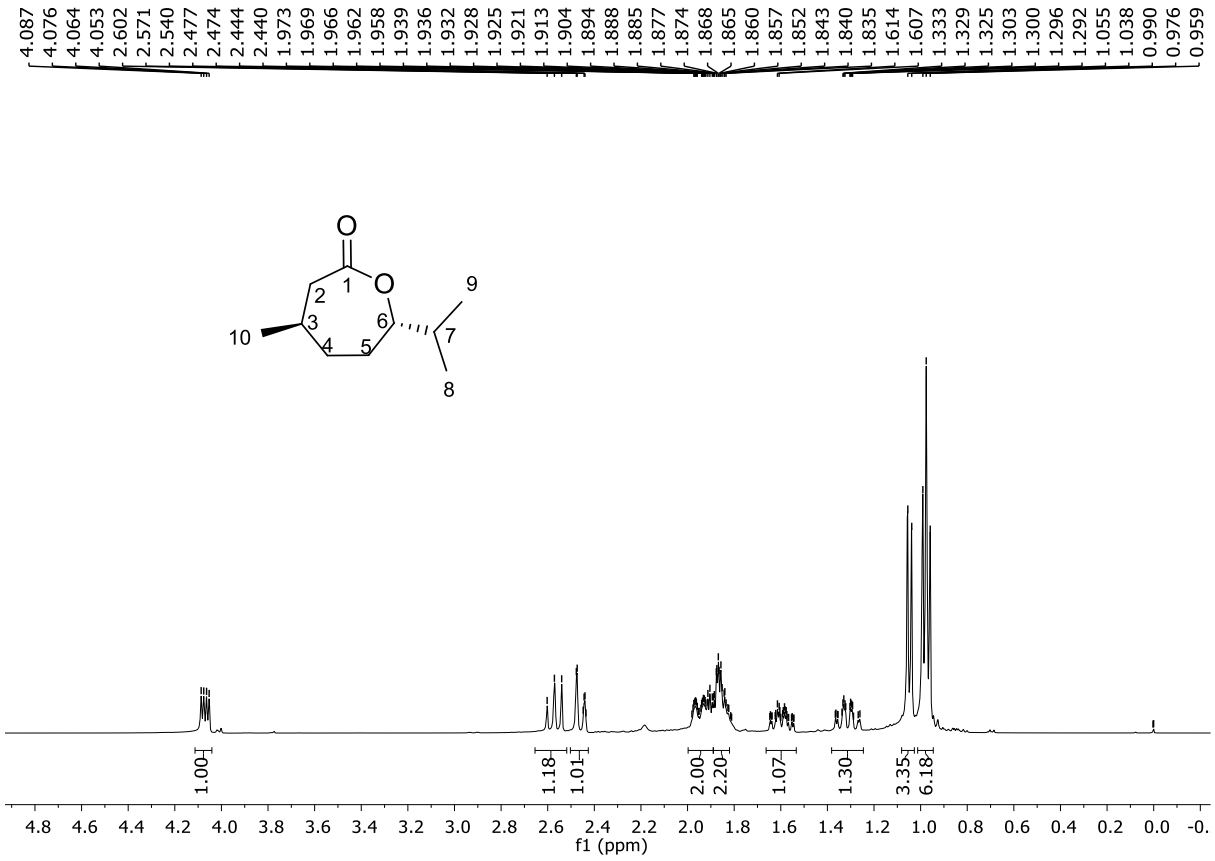


Figure S17. ^{1}H NMR for compound 4 ($CDCl_3$, 400 MHz)

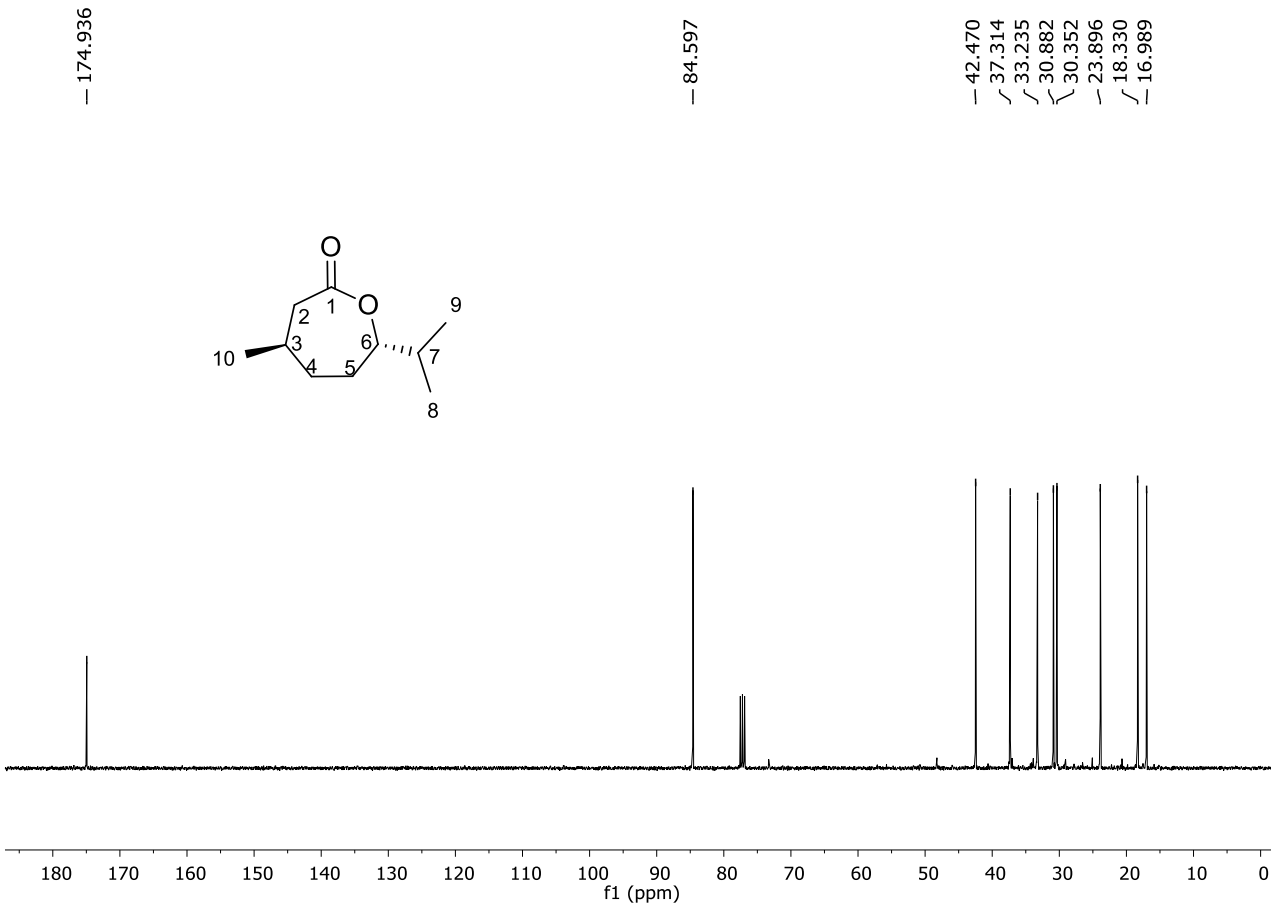


Figure S18. ^{13}C APT NMR for compound 4 ($CDCl_3$, 101 MHz)

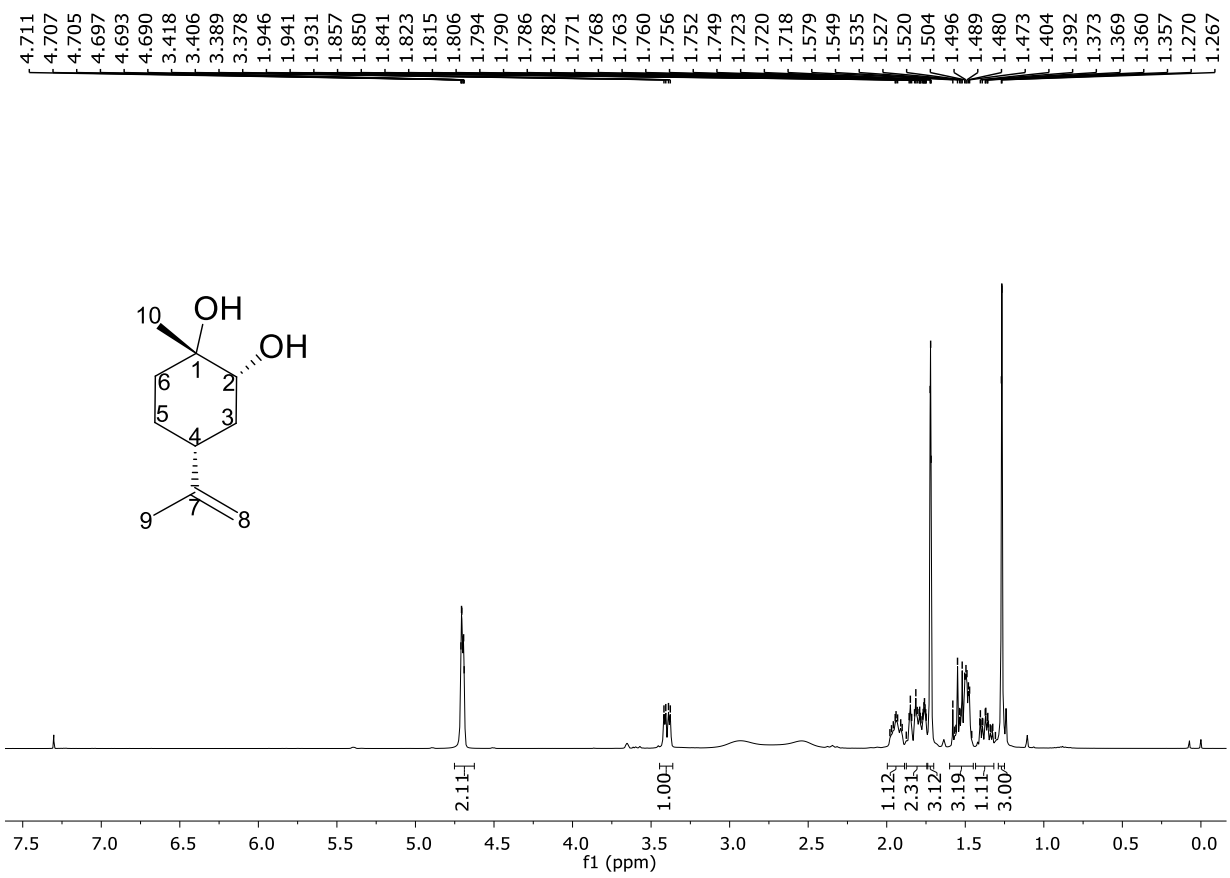


Figure S19. ^{1}H NMR for compound 5a ($CDCl_3$, 400 MHz)

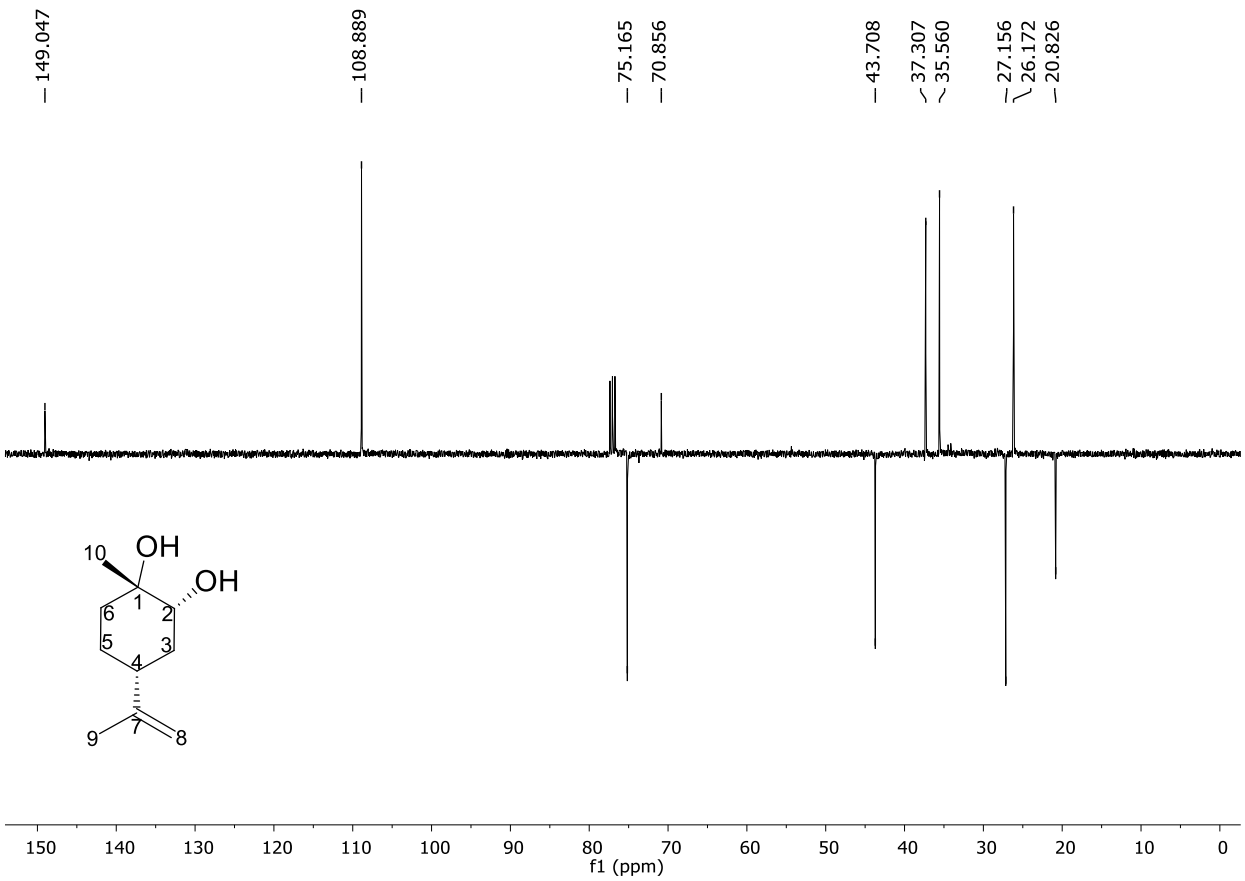


Figure S20. ^{13}C APT NMR for compound 5a ($CDCl_3$, 101 MHz)

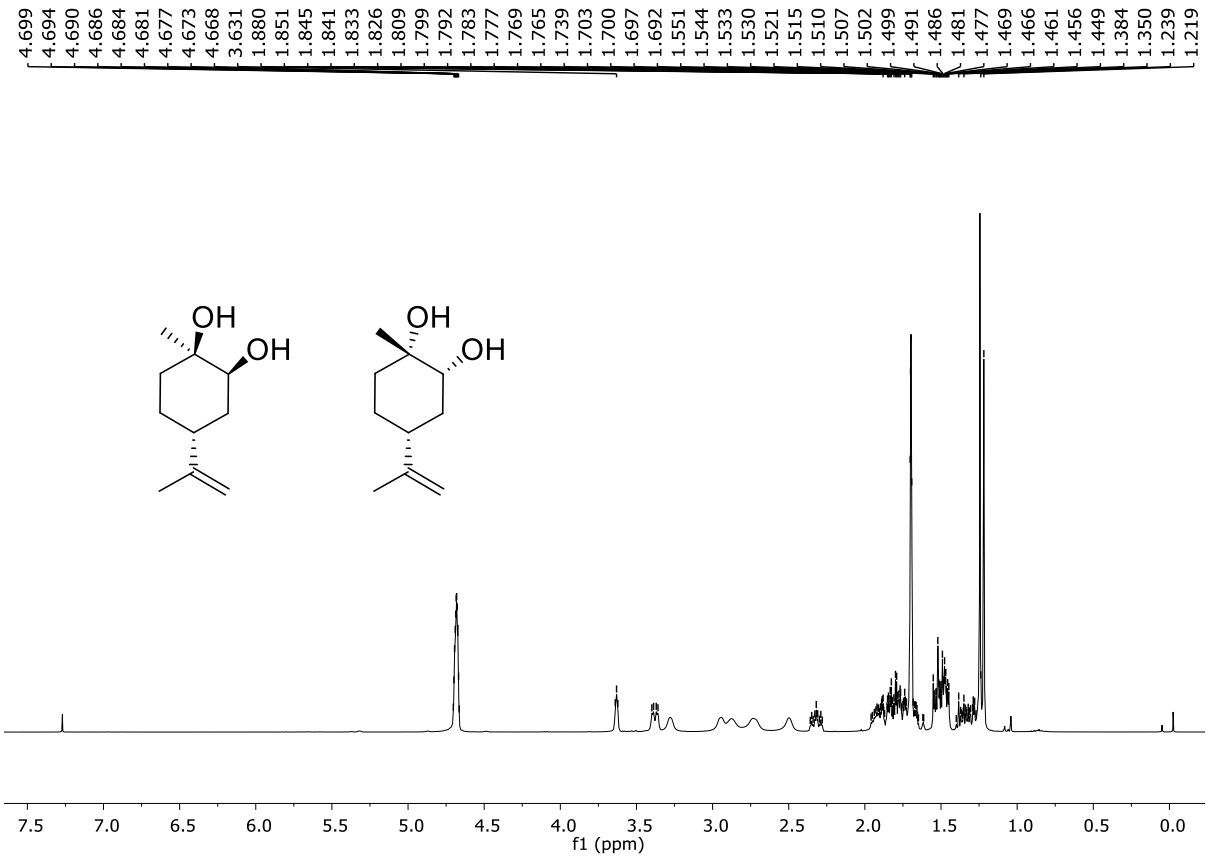


Figure S21. ^{1}H NMR for mixture 5b/5a ($CDCl_3$, 400 MHz)

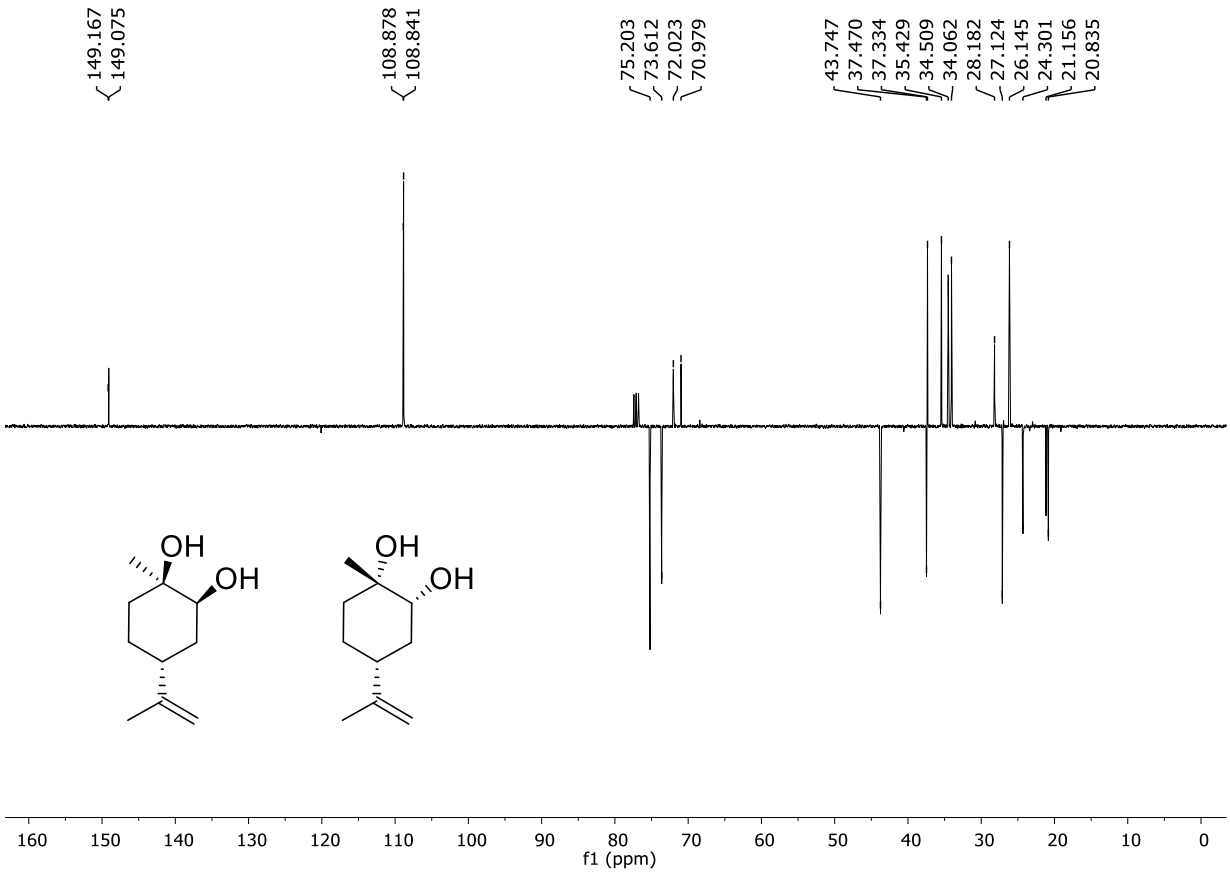


Figure S22. ^{13}C APT NMR for mixture 5b/5a ($CDCl_3$, 101 MHz)

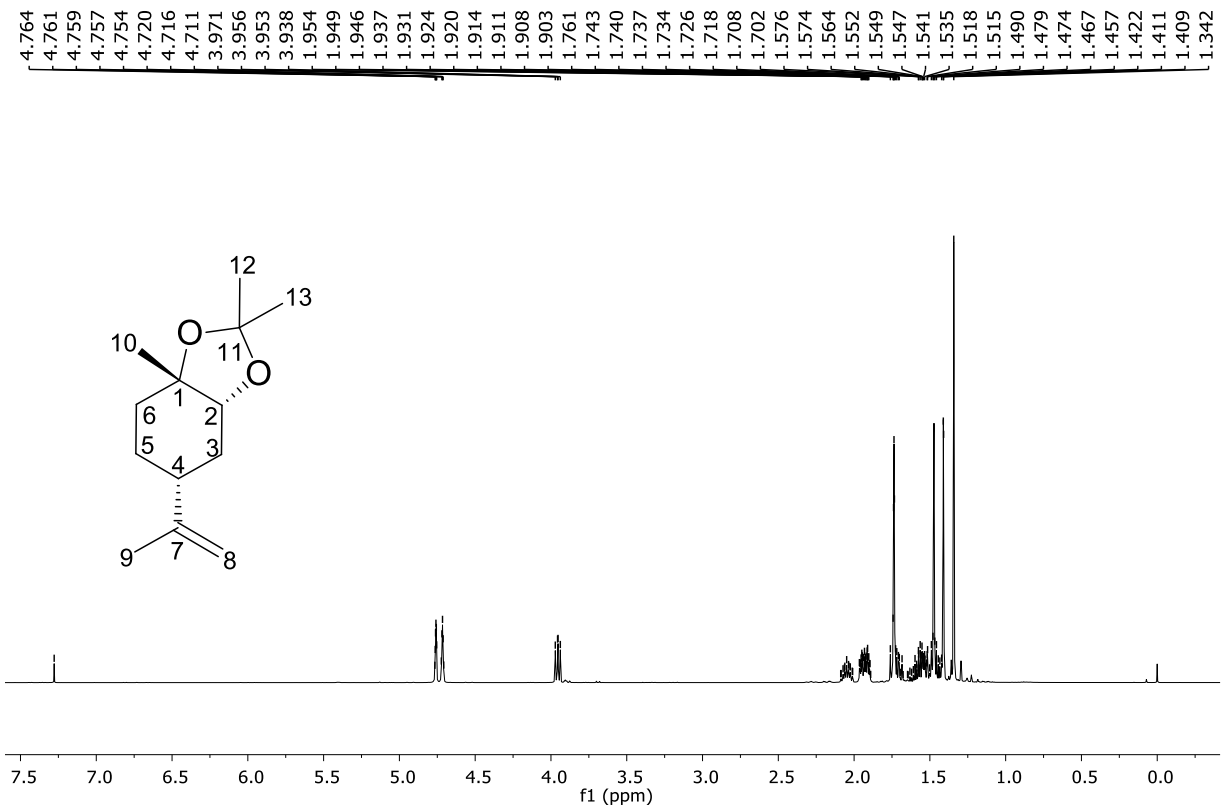


Figure S23. ^{1}H NMR for compound 6a ($CDCl_3$, 400 MHz)

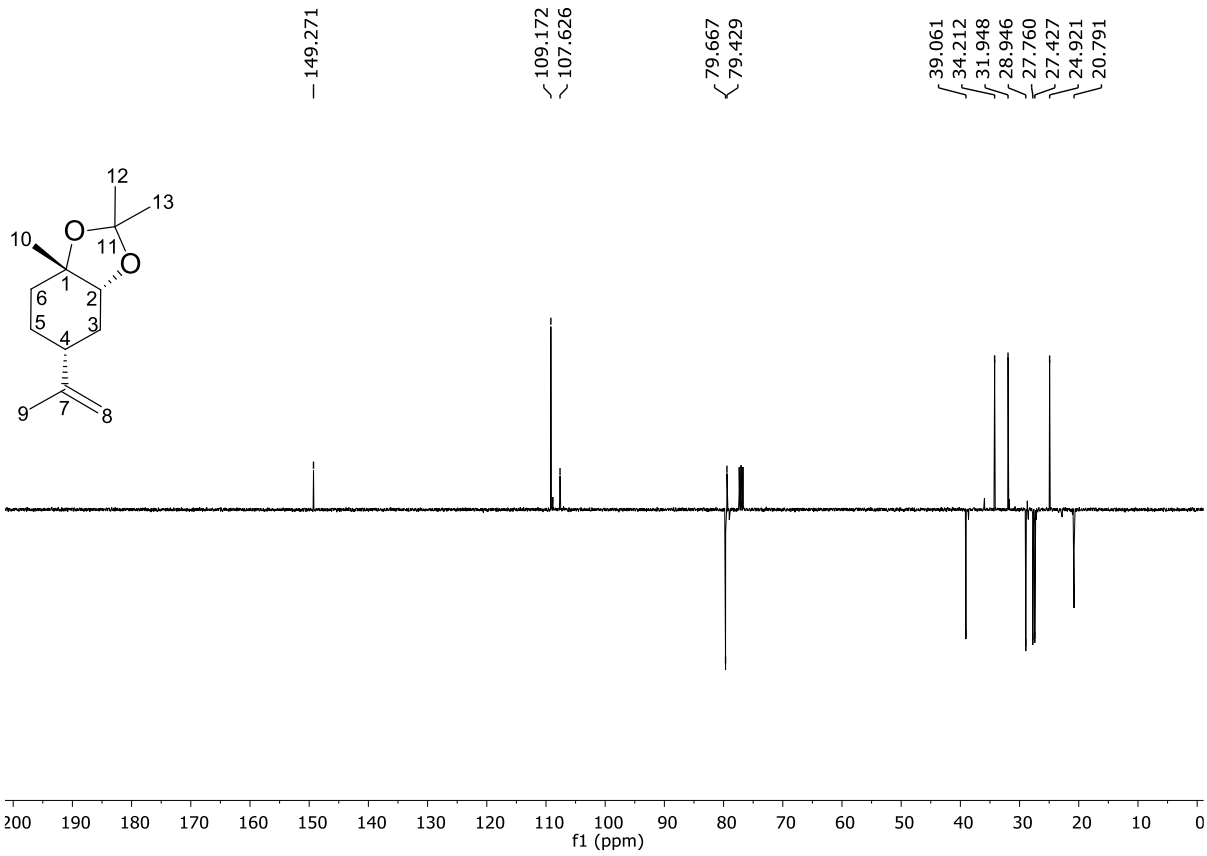


Figure S24. ^{13}C APT NMR for compound 6a ($CDCl_3$, 101 MHz)

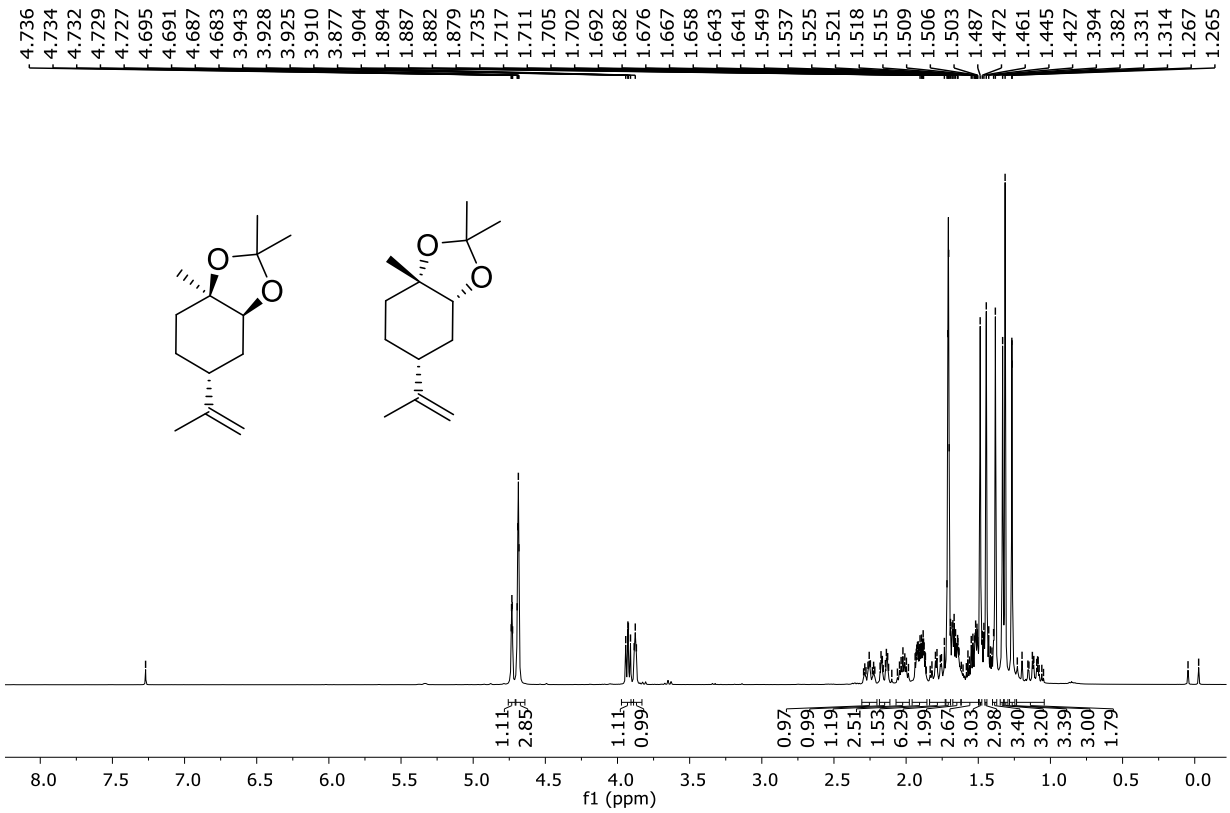


Figure S25. ^{1}H NMR for compound 6b ($CDCl_3$, 400 MHz)

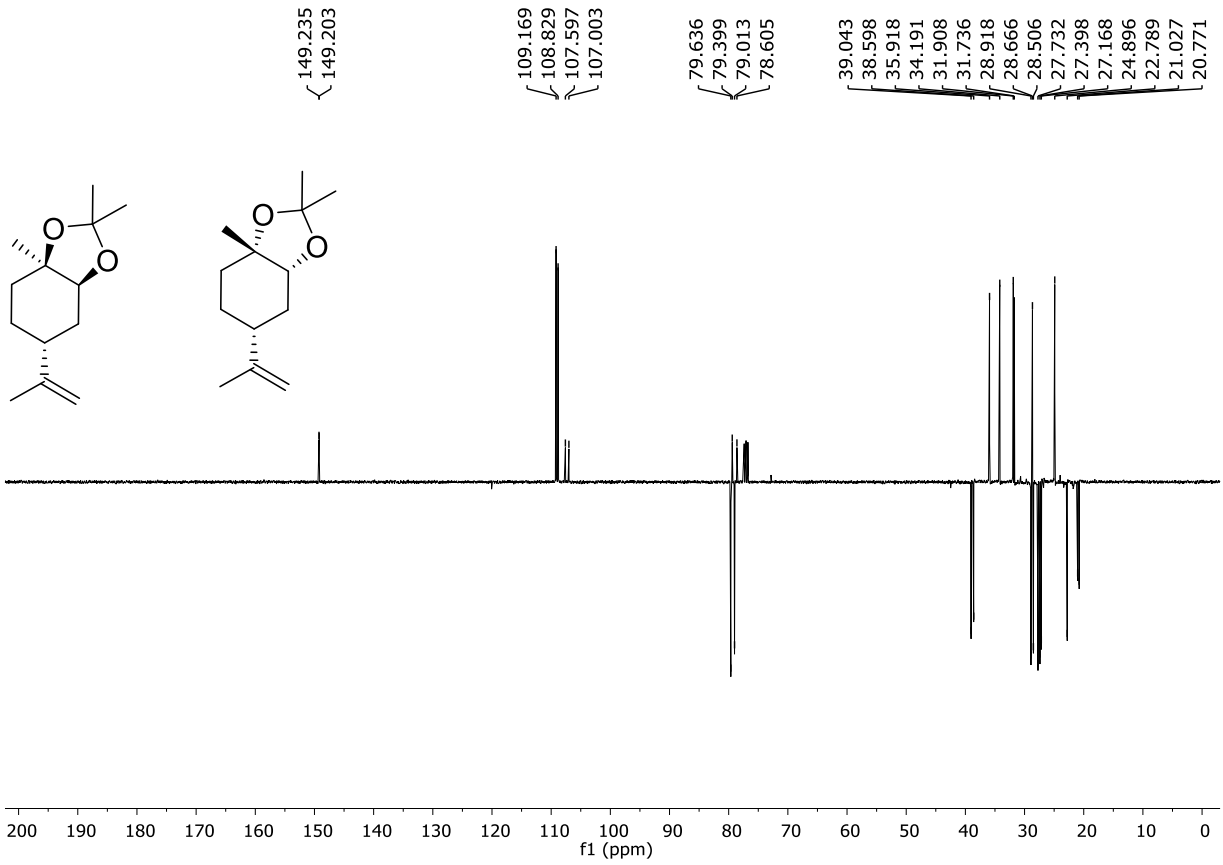


Figure S26. ^{13}C APT NMR for compound 6b ($CDCl_3$, 101 MHz)

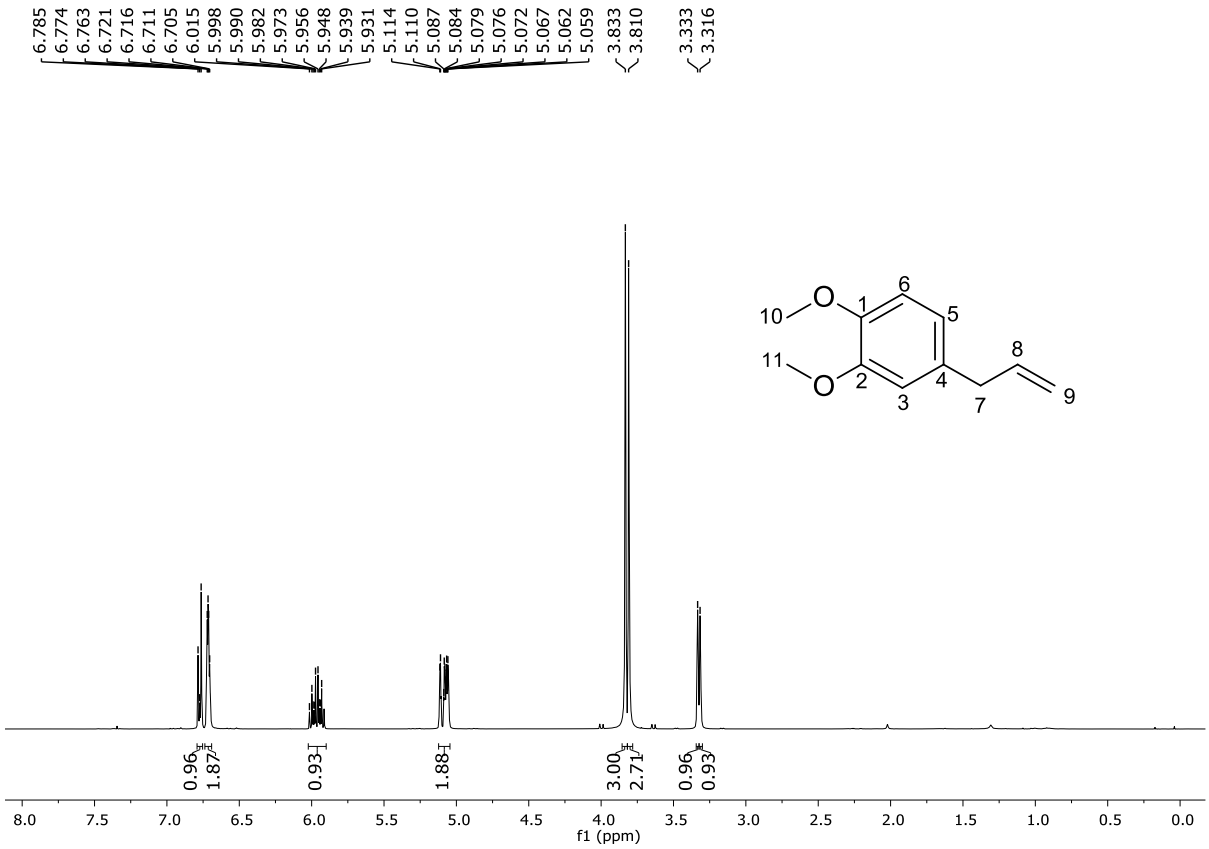


Figure S27. ^{1}H NMR for compound CH8 ($CDCl_3$, 400 MHz)

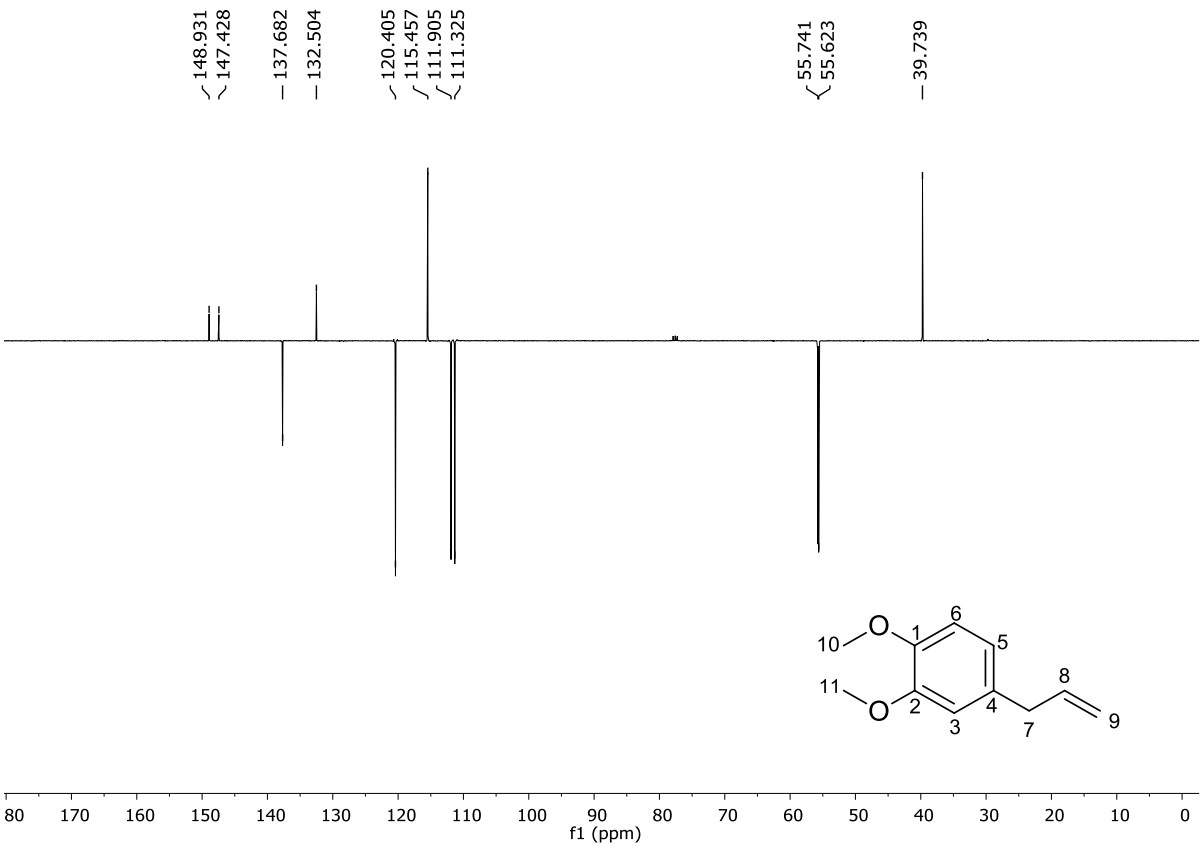


Figure S28. ^{13}C APT NMR for compound CH8 ($CDCl_3$, 101 MHz)

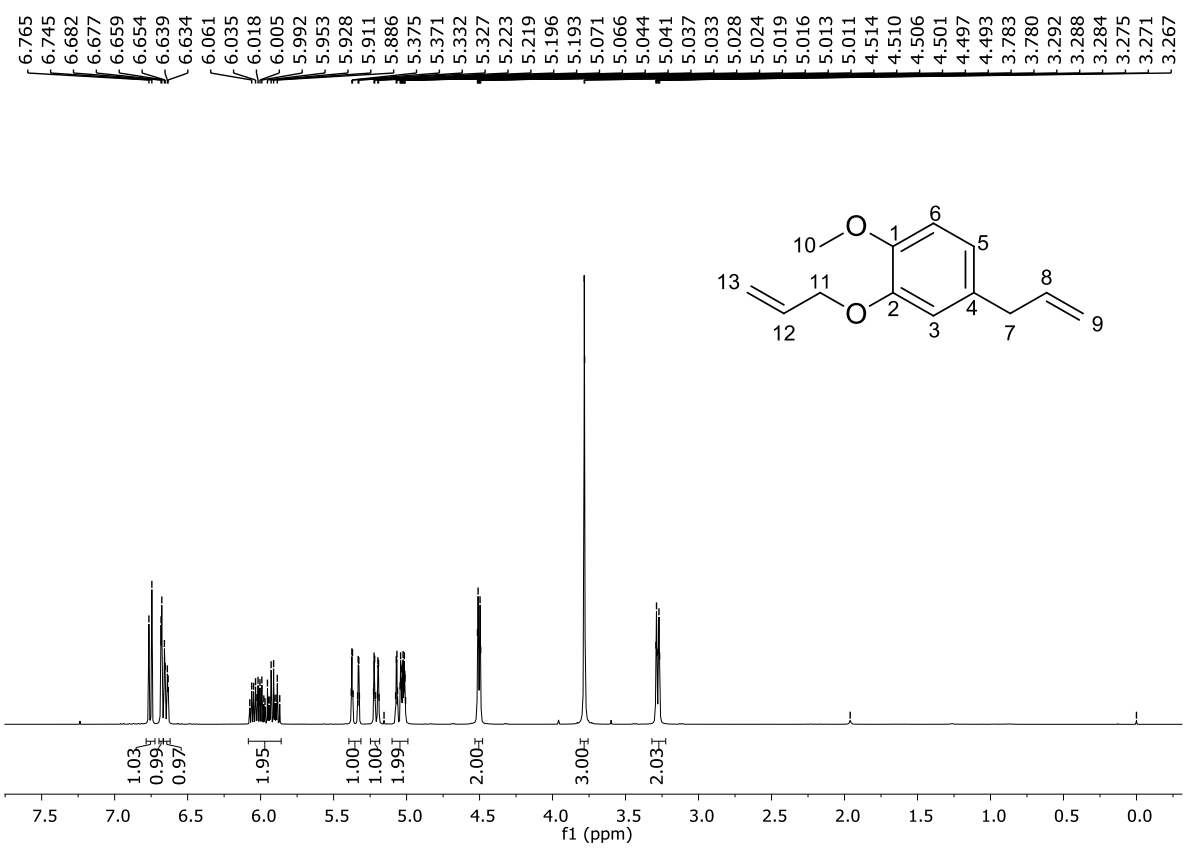


Figure S29. ^{1}H NMR for compound 7a ($CDCl_3$, 400 MHz)

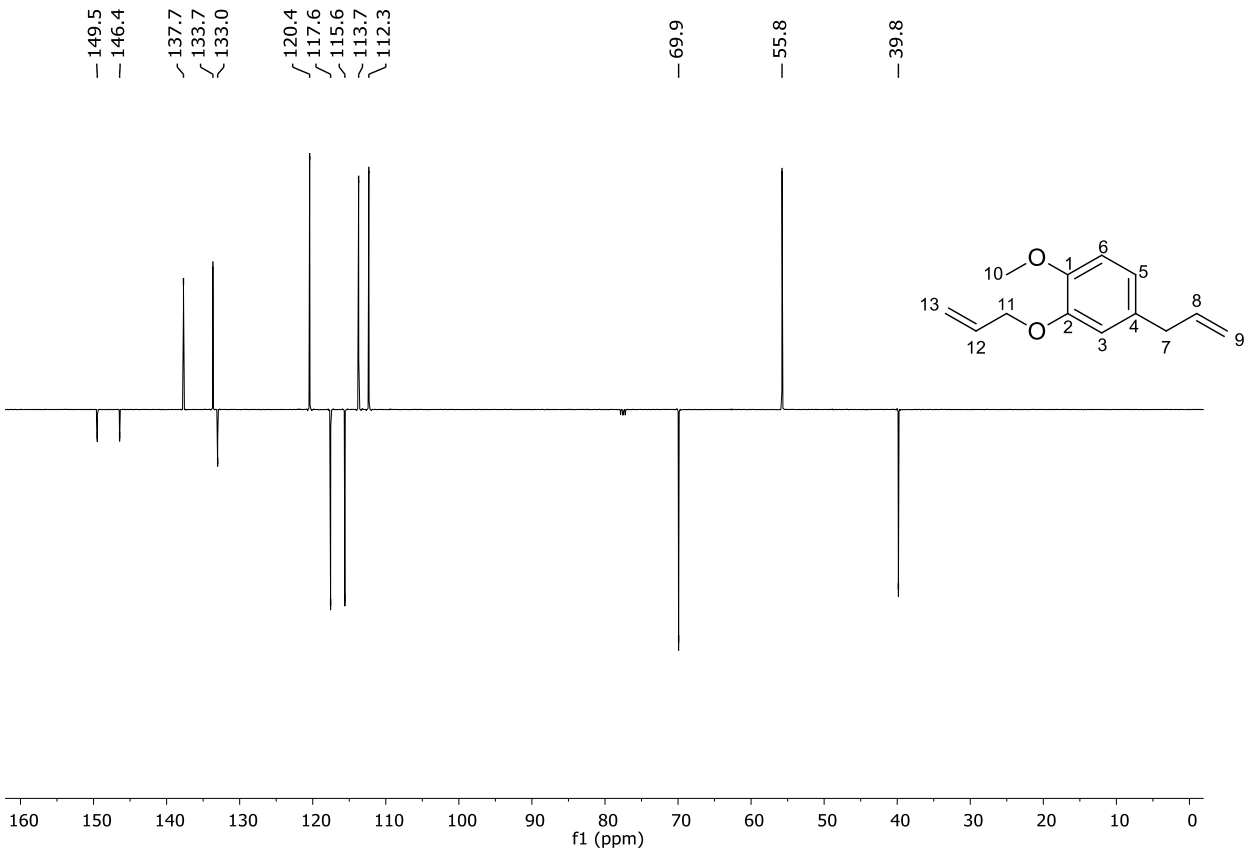


Figure S30. ^{13}C APT NMR for compound 7a ($CDCl_3$, 101 MHz)

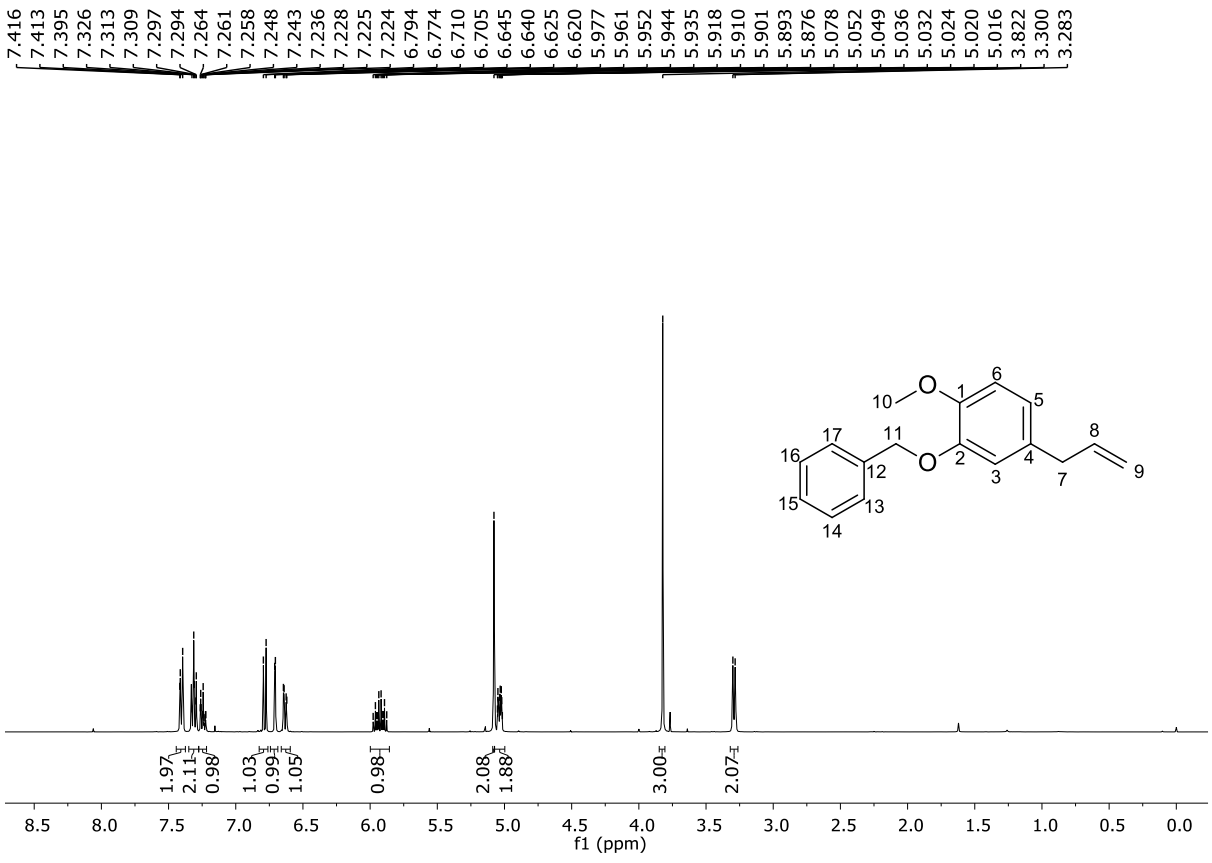


Figure S31. ^{1}H NMR for compound 7b ($CDCl_3$, 400 MHz)

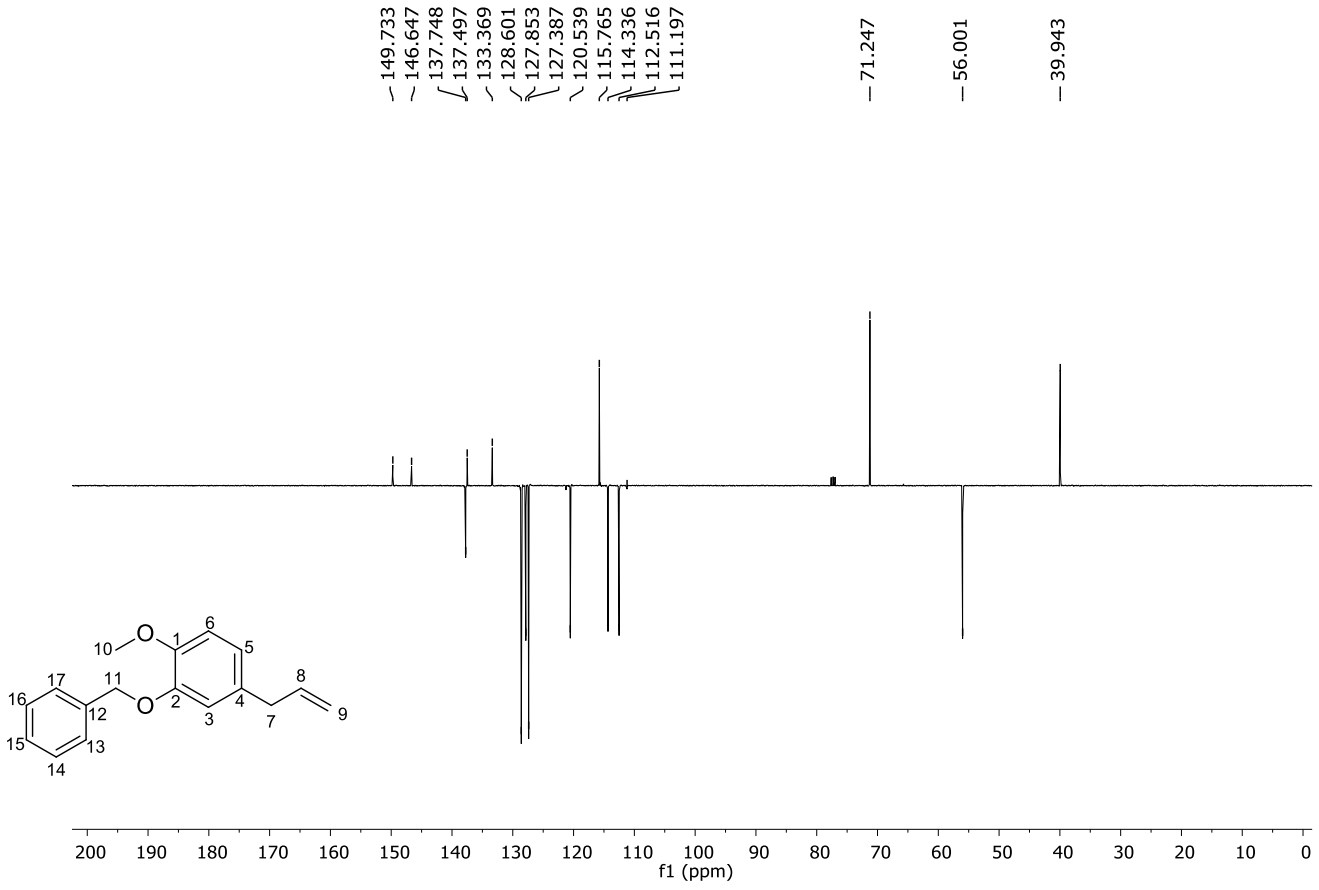


Figure S32. ^{13}C APT NMR for compound 7b ($CDCl_3$, 101 MHz)

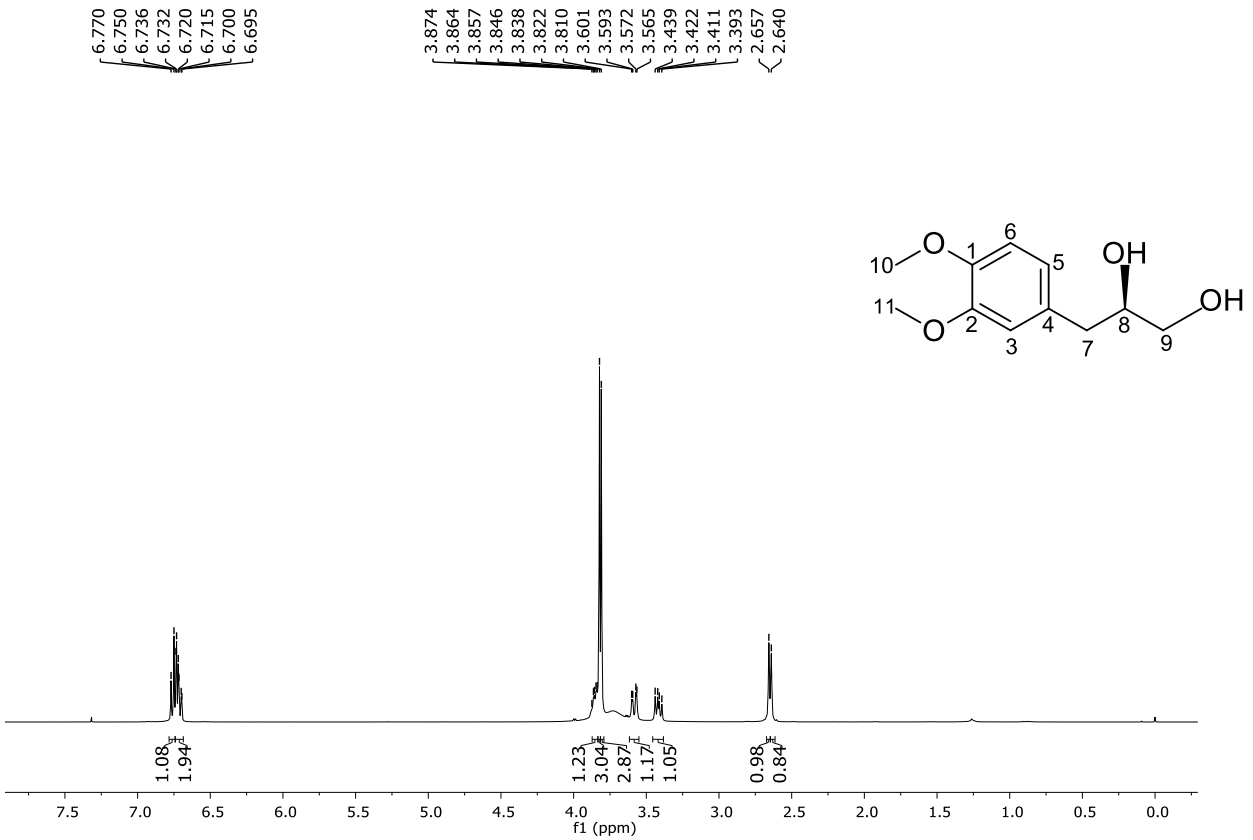


Figure S33. ^{1}H NMR for compound 8a ($CDCl_3$, 400 MHz)



Figure S34. ^{13}C APT NMR for compound 8a. ($CDCl_3$, 101 MHz)

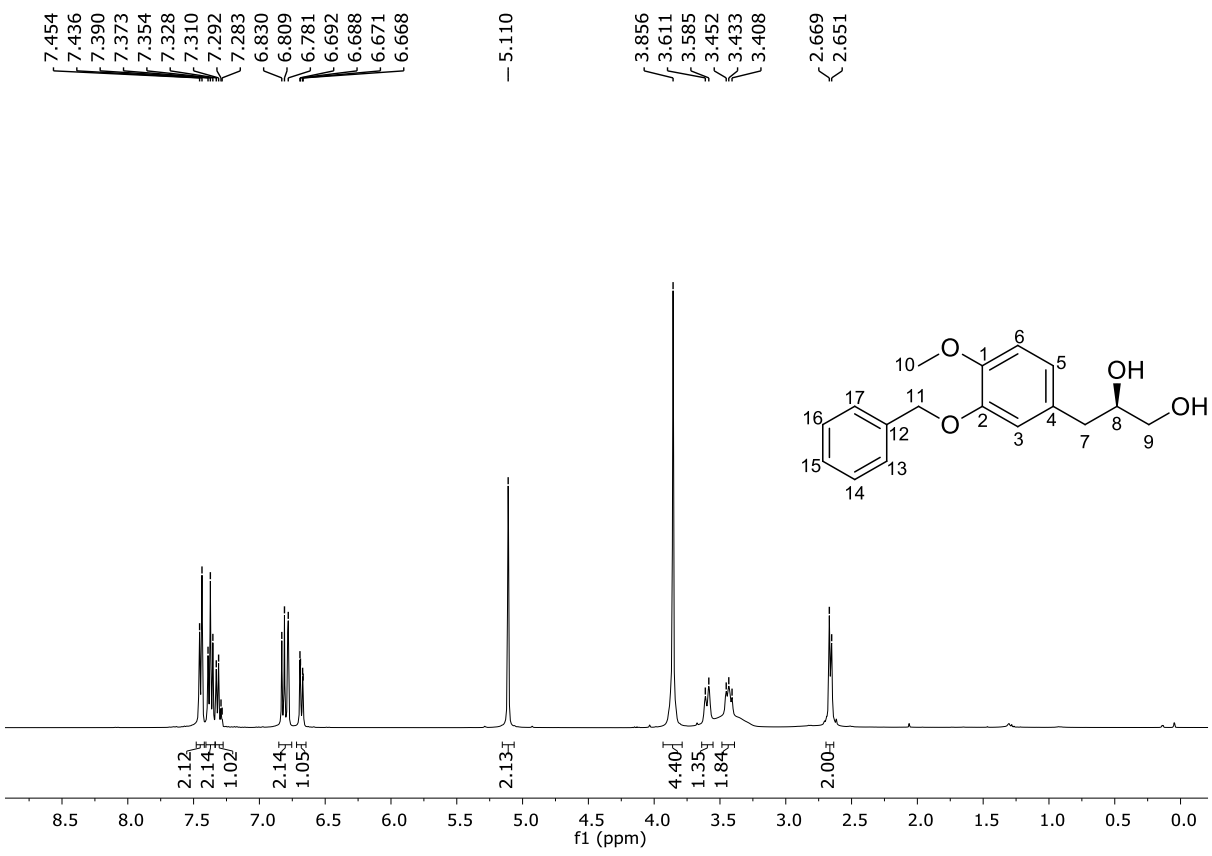


Figure S35. ^{1}H NMR for compound 8b ($CDCl_3$, 400 MHz)

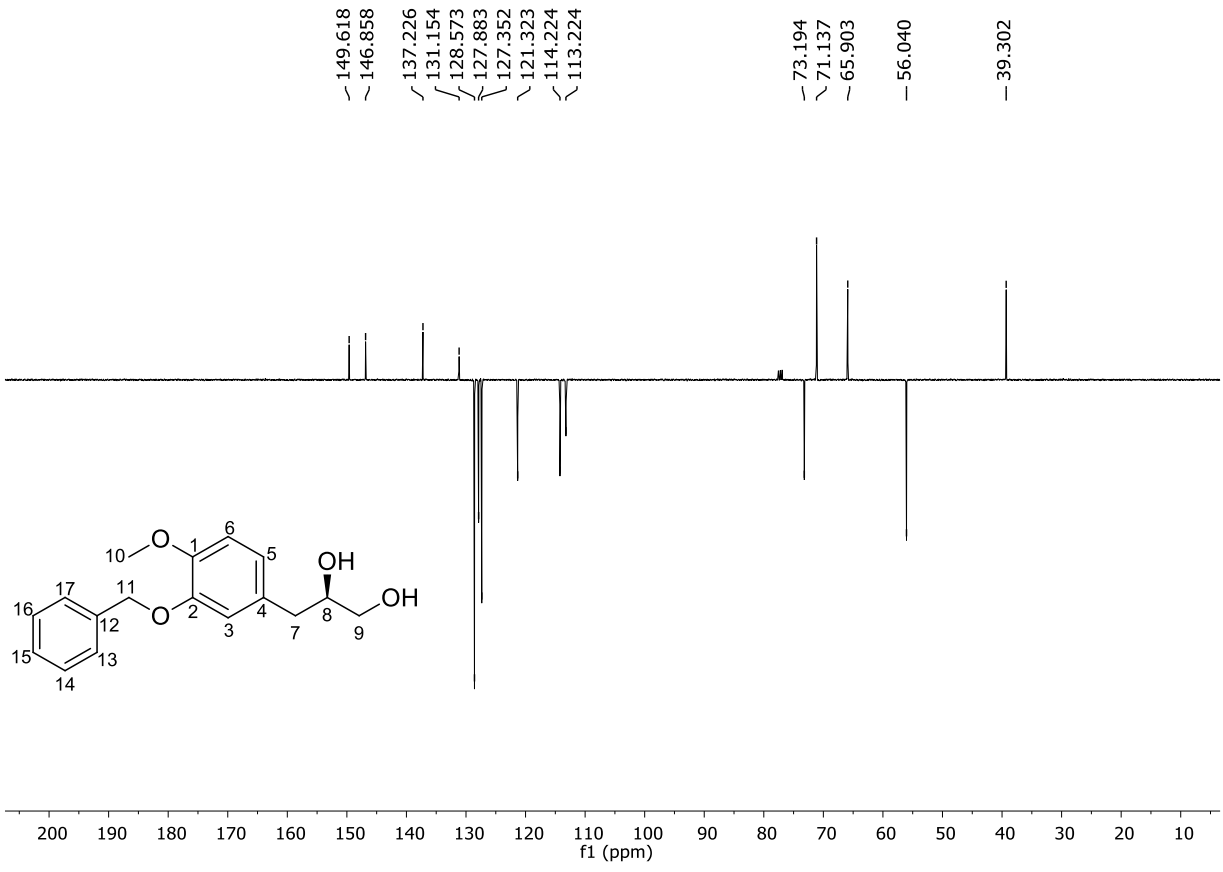


Figure S36. ^{13}C APT NMR for compound 8b ($CDCl_3$, 101 MHz)

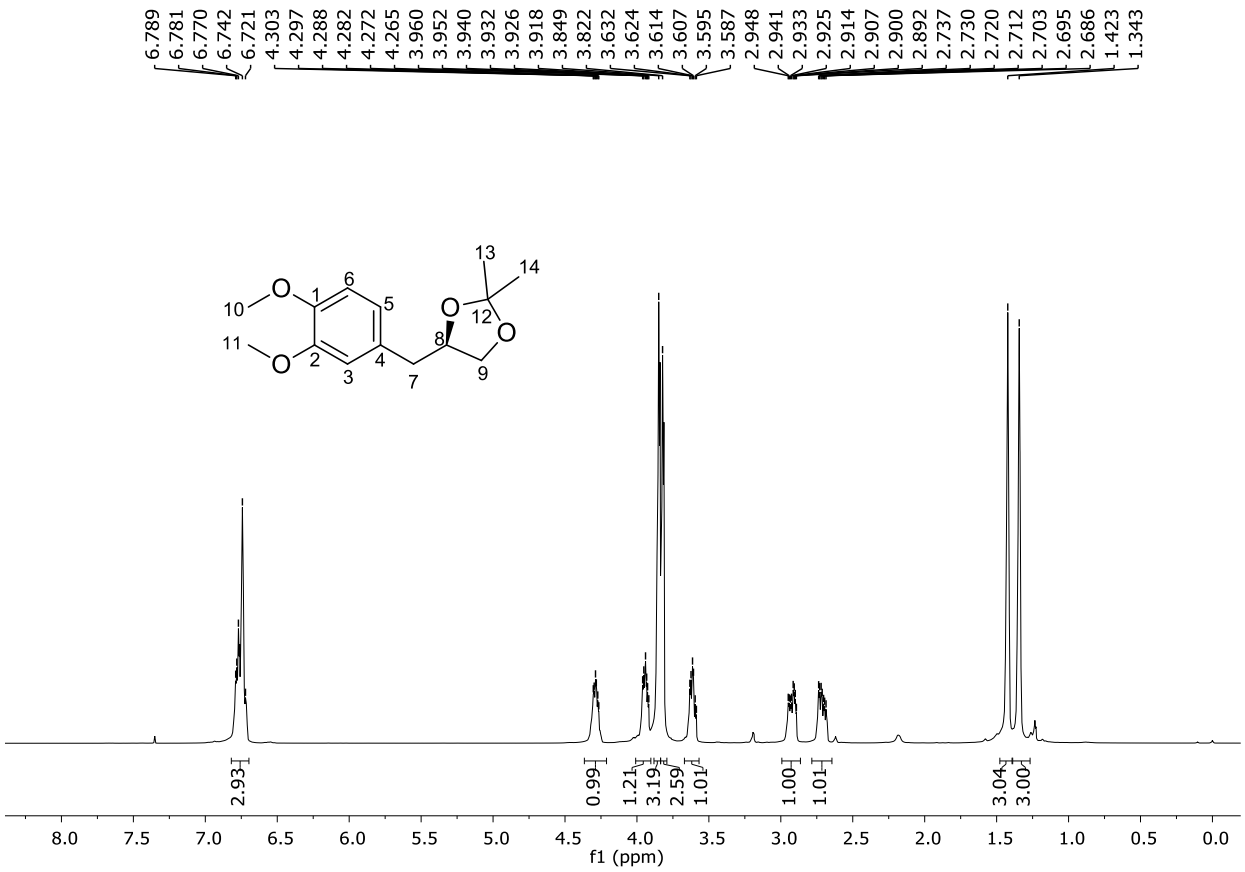


Figure S37. ^{1}H NMR for compound 9a ($CDCl_3$, 400 MHz)

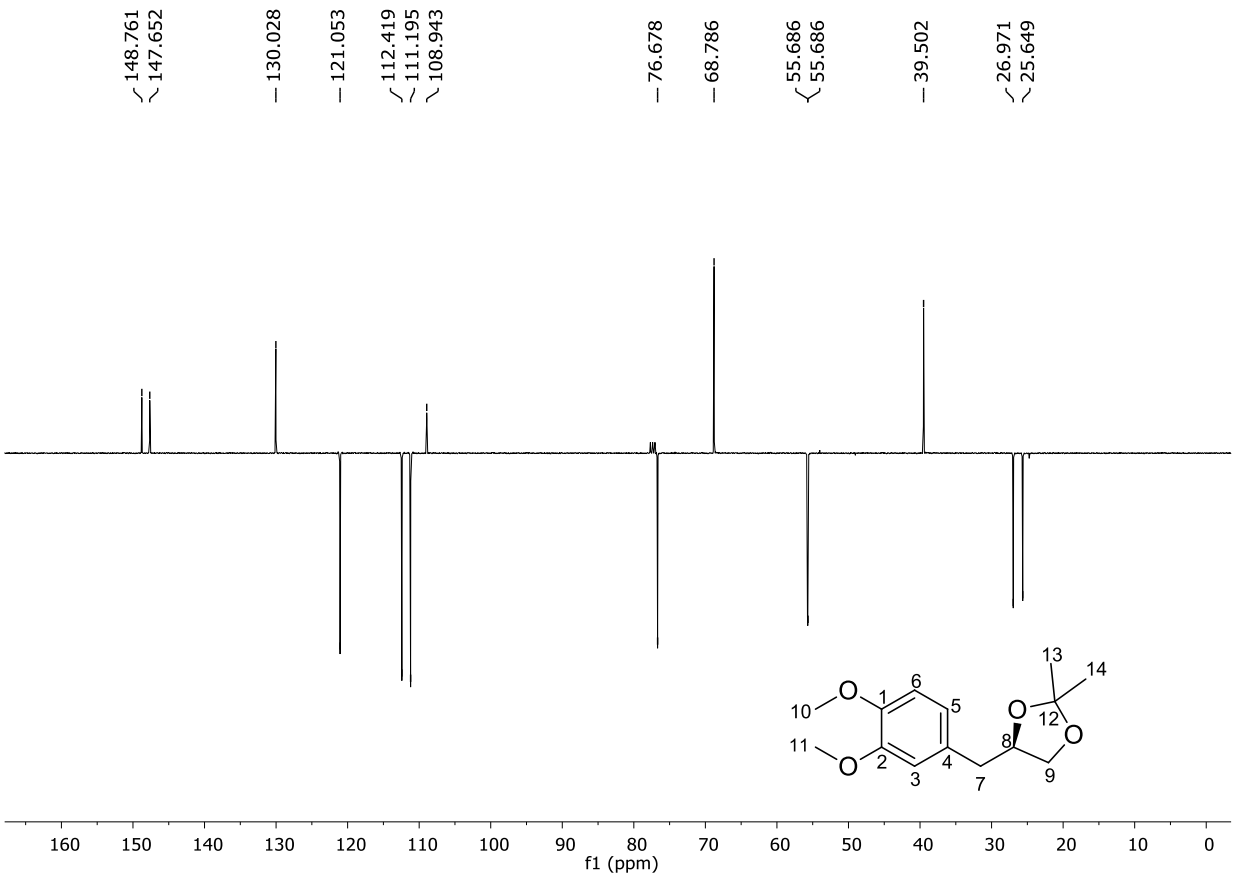


Figure S38. ^{13}C APT NMR for compound 9a ($CDCl_3$, 101 MHz)

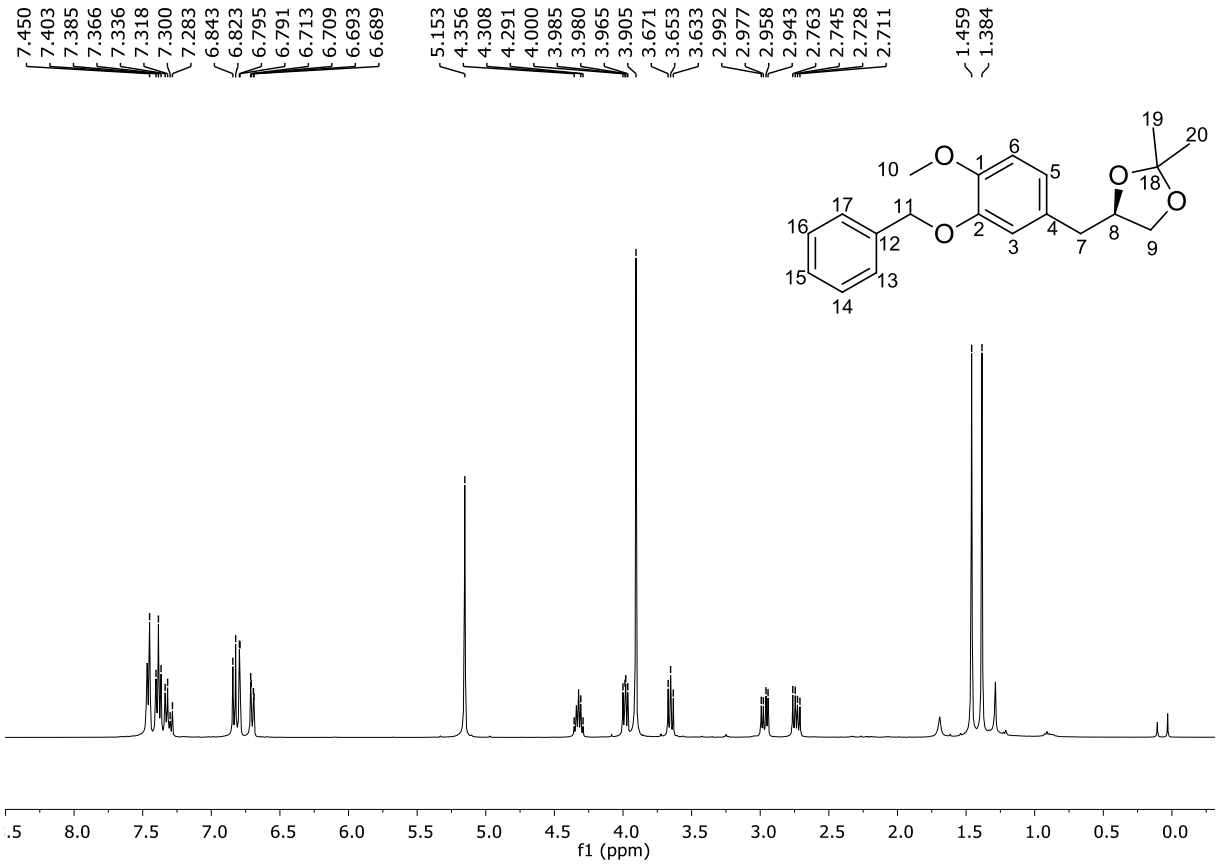


Figure S39. ^{1}H NMR for compound 9b ($CDCl_3$, 400 MHz)

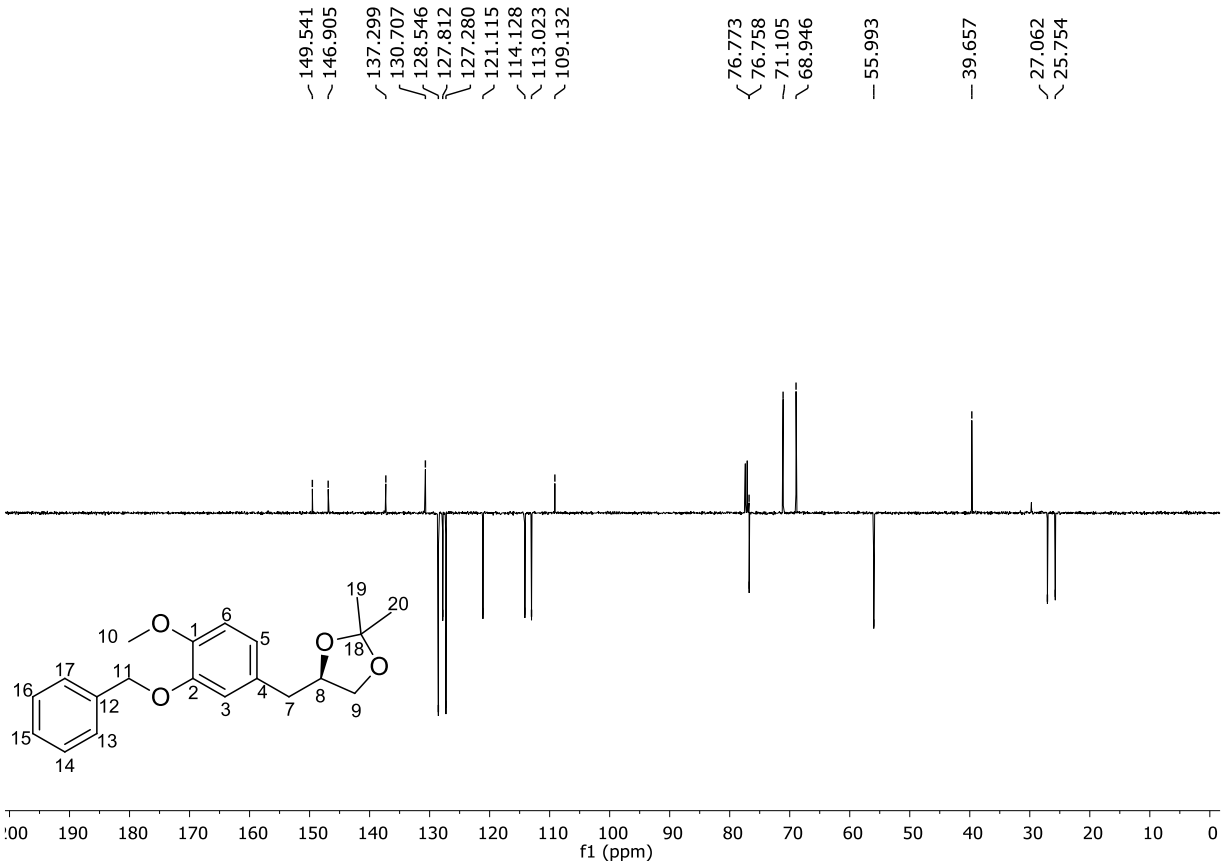


Figure S40. ^{13}C APT NMR for compound 9b ($CDCl_3$, 101 MHz)

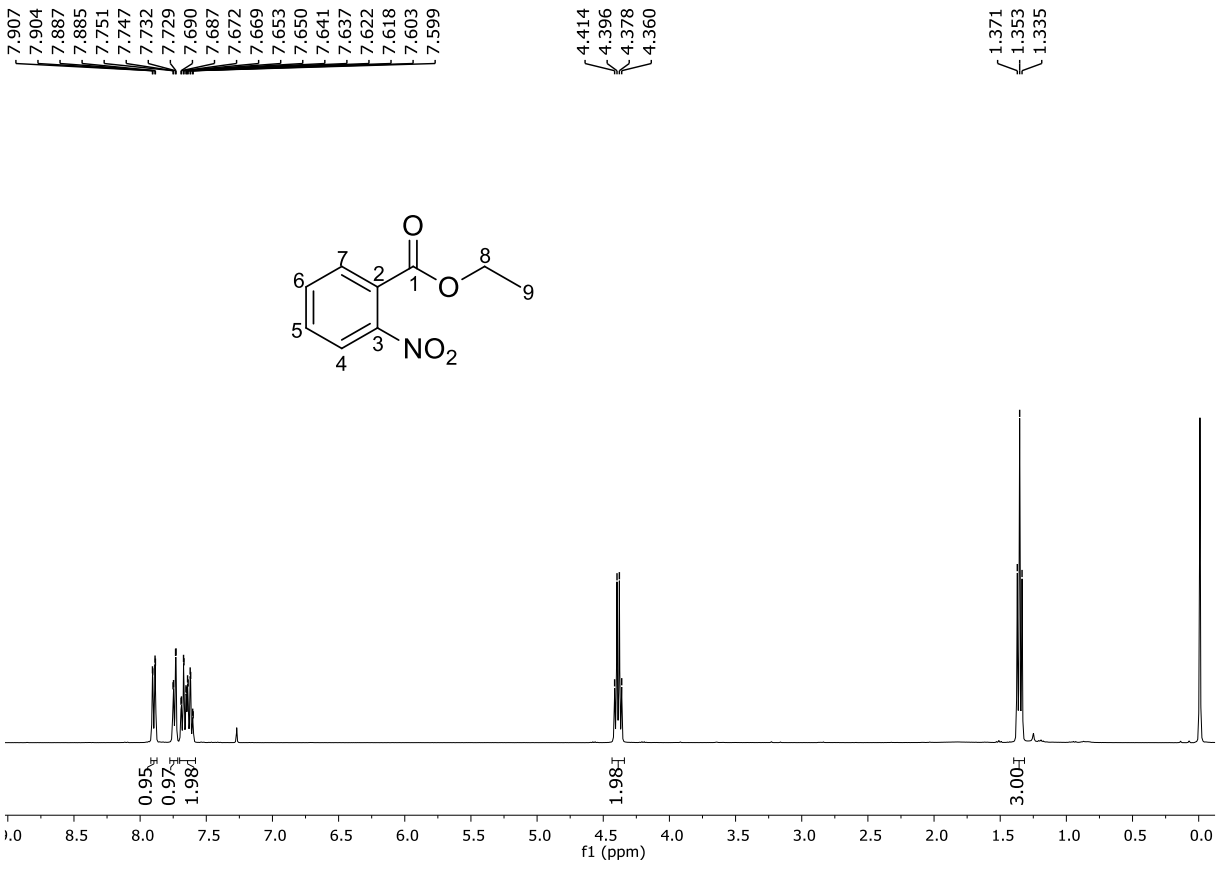


Figure S41. ^{1}H NMR for compound 10a ($CDCl_3$, 400 MHz)

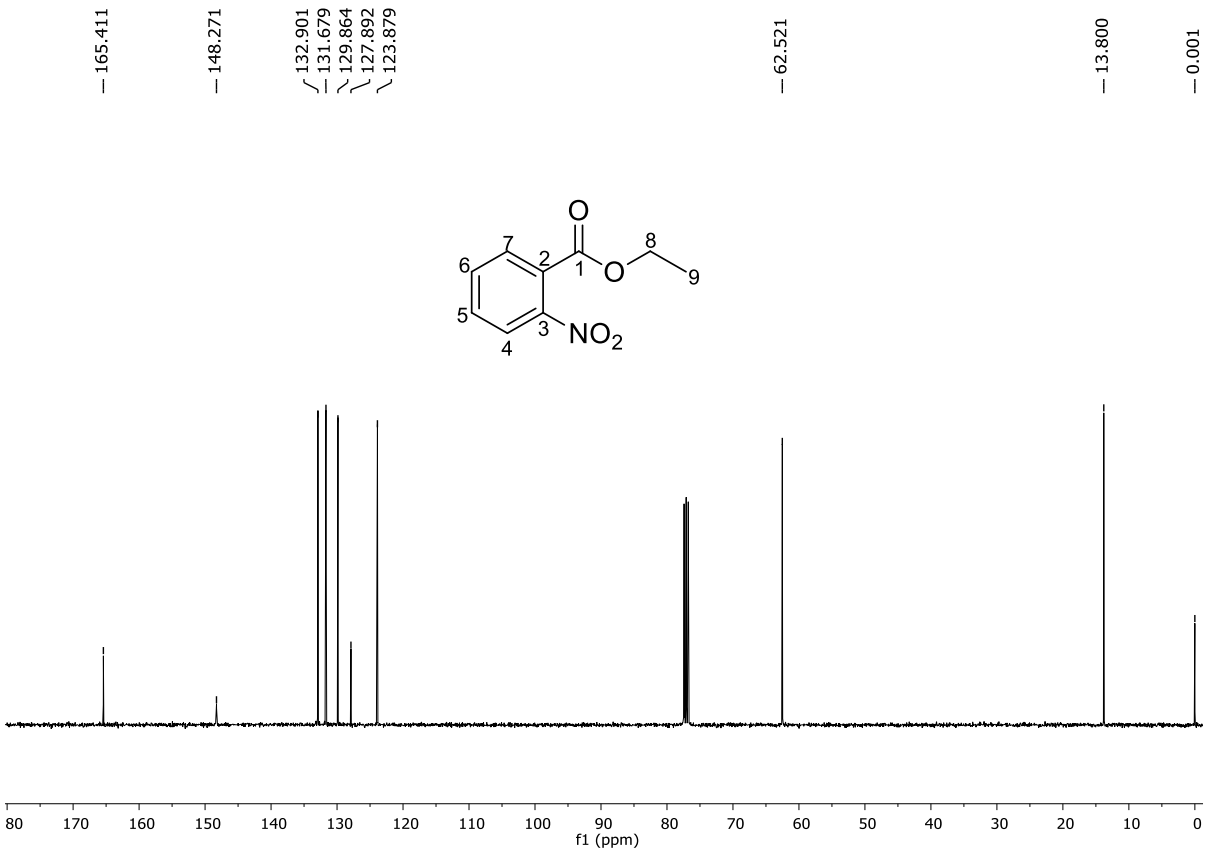


Figure S42. ^{13}C NMR for compound 10a ($CDCl_3$, 101 MHz)

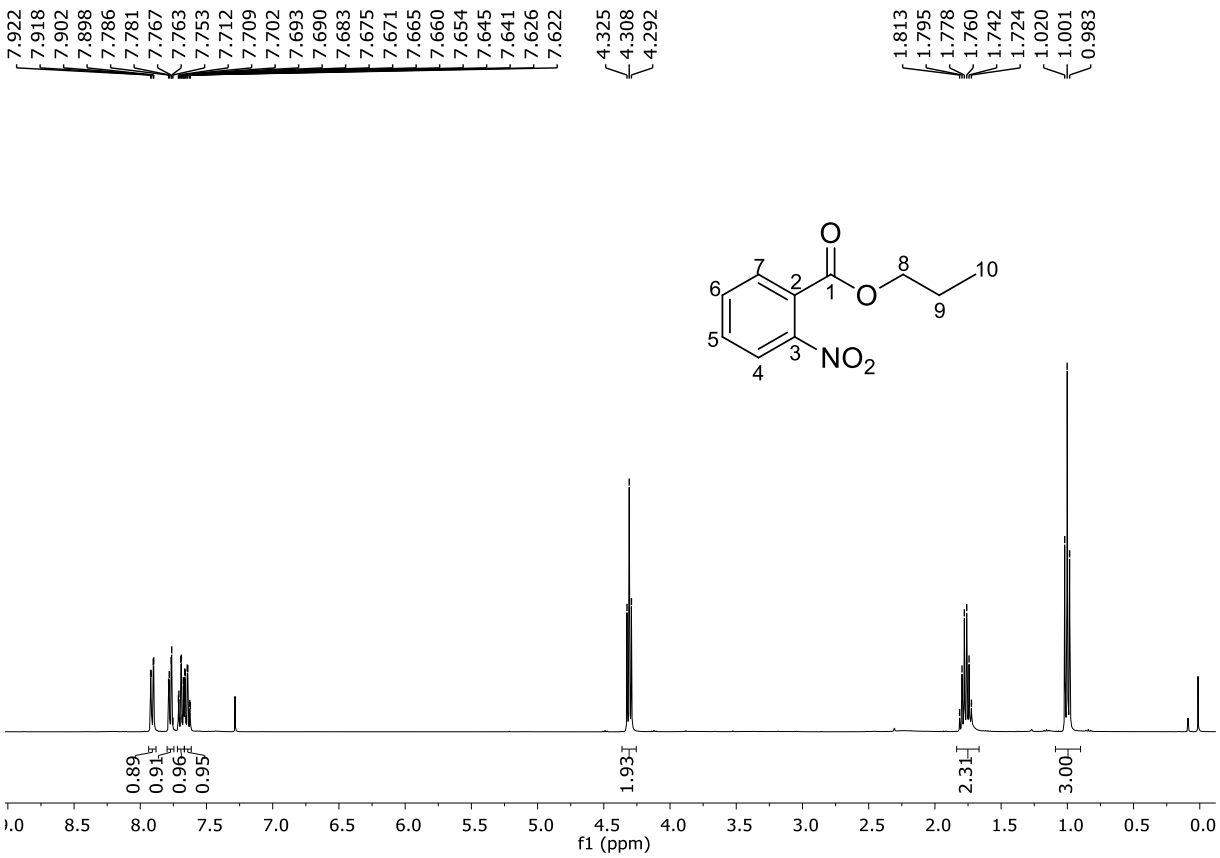


Figure S43. ^{1}H NMR for compound 10b ($CDCl_3$, 400 MHz)

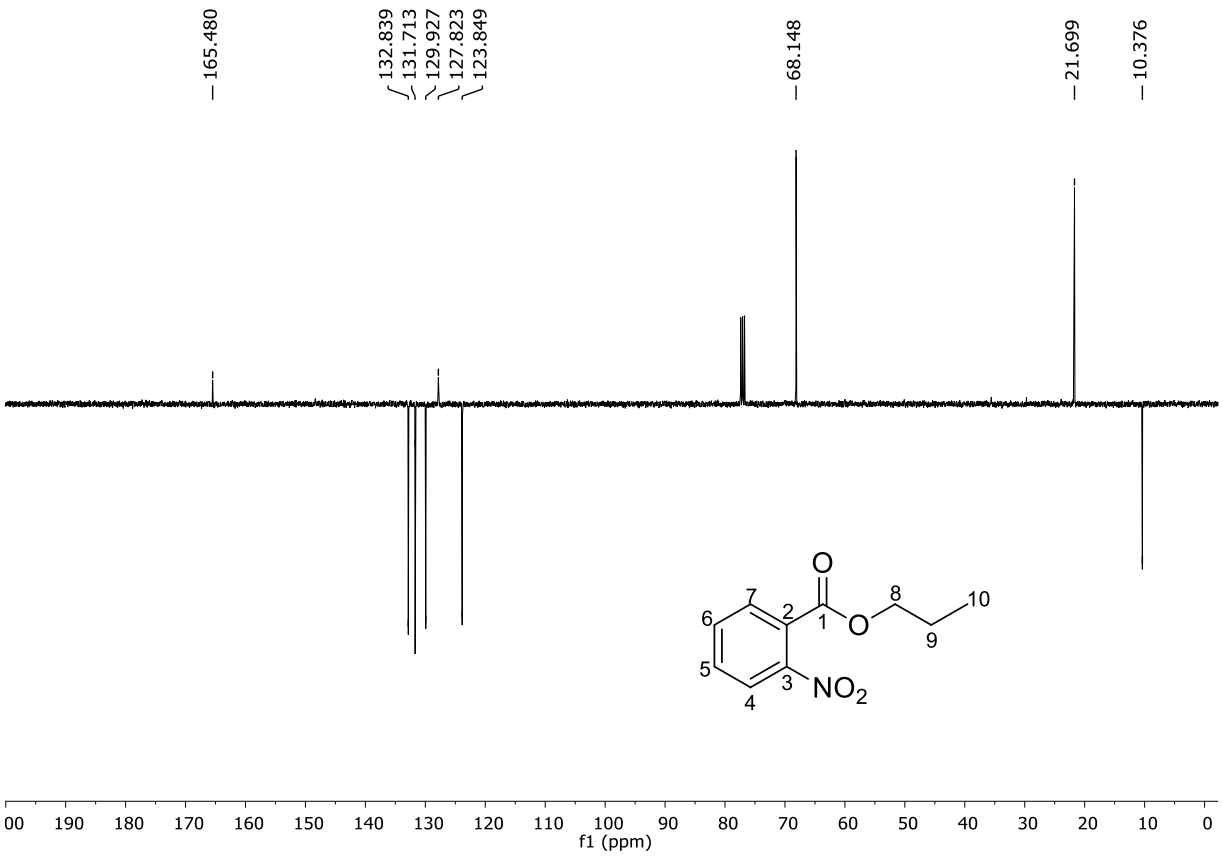


Figure S44. ^{13}C NMR APT for compound 10b ($CDCl_3$, 101 MHz)

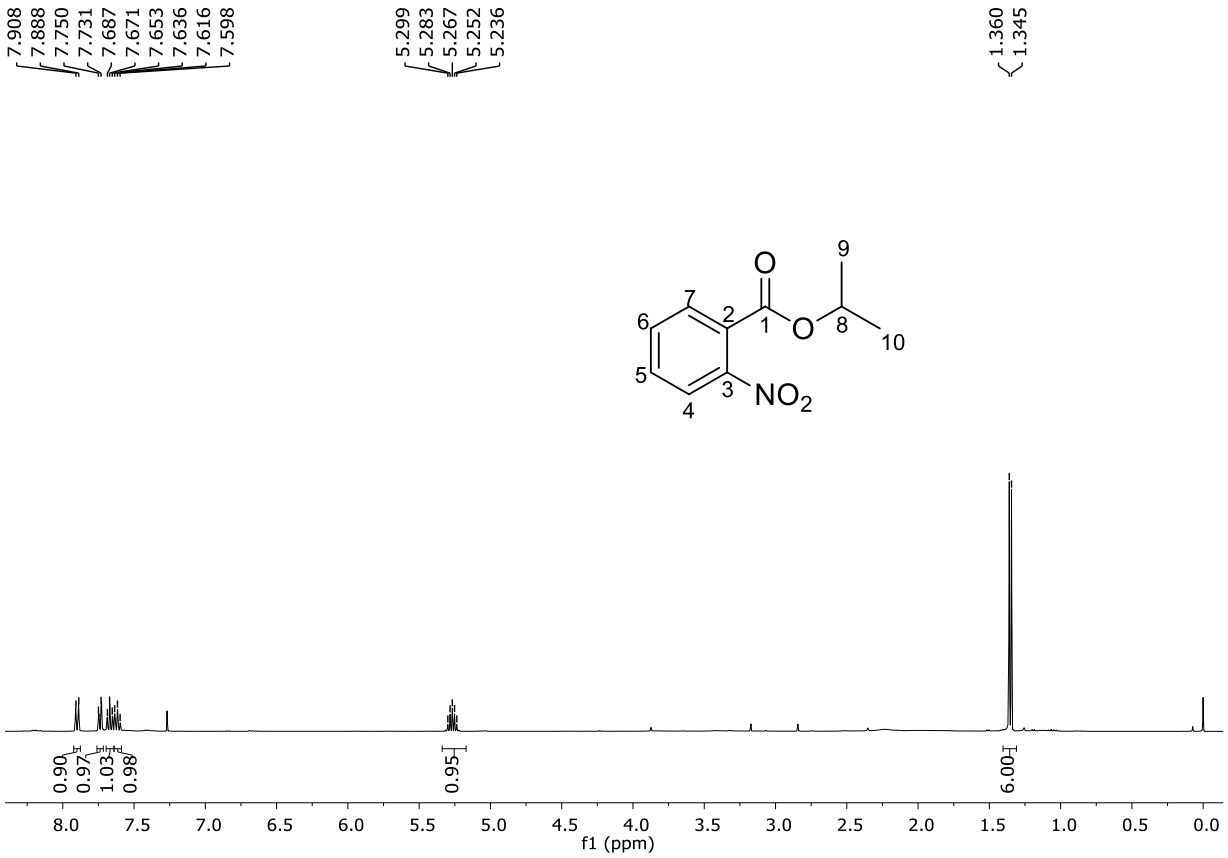


Figure S45. ^{1}H NMR for compound 10c ($CDCl_3$, 400 MHz)

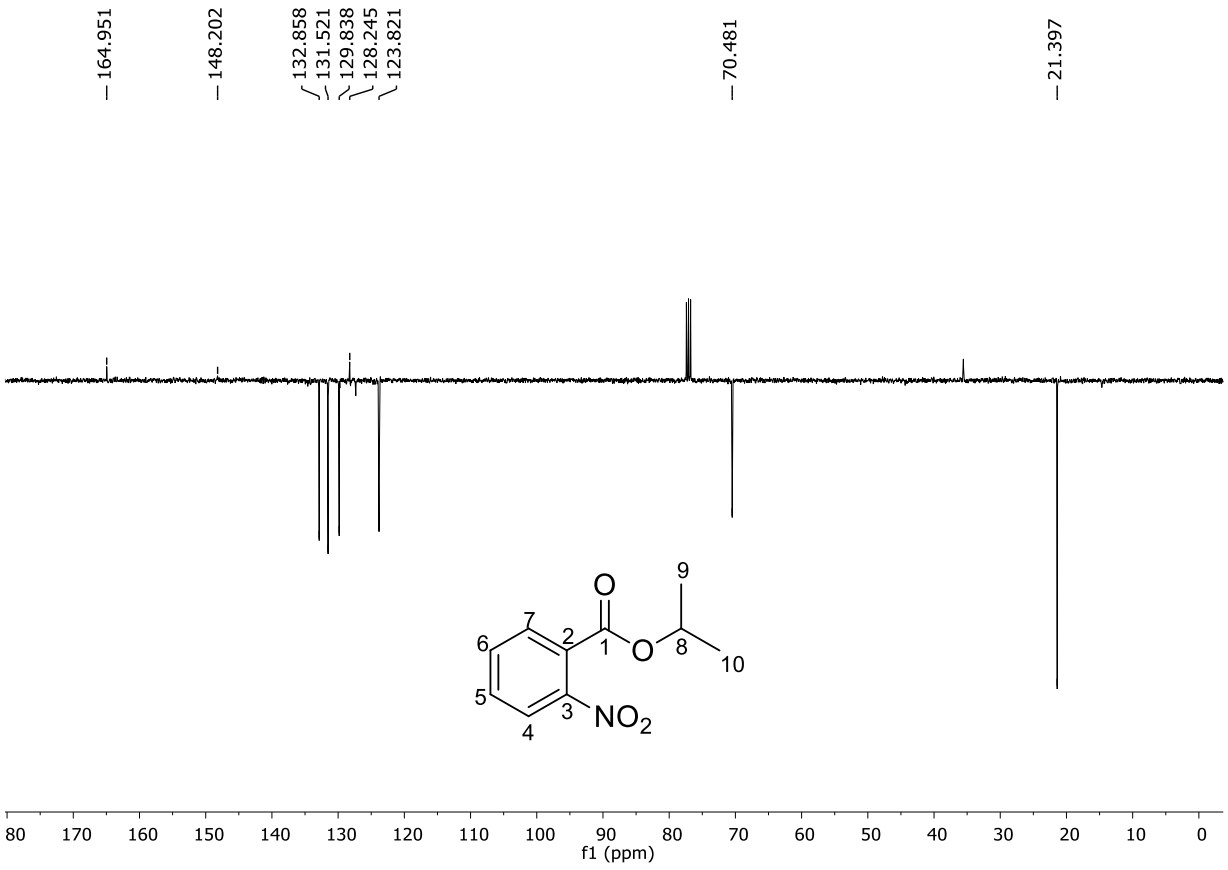


Figura S46. ^{13}C NMR for compound 10c ($CDCl_3$, 101 MHz)

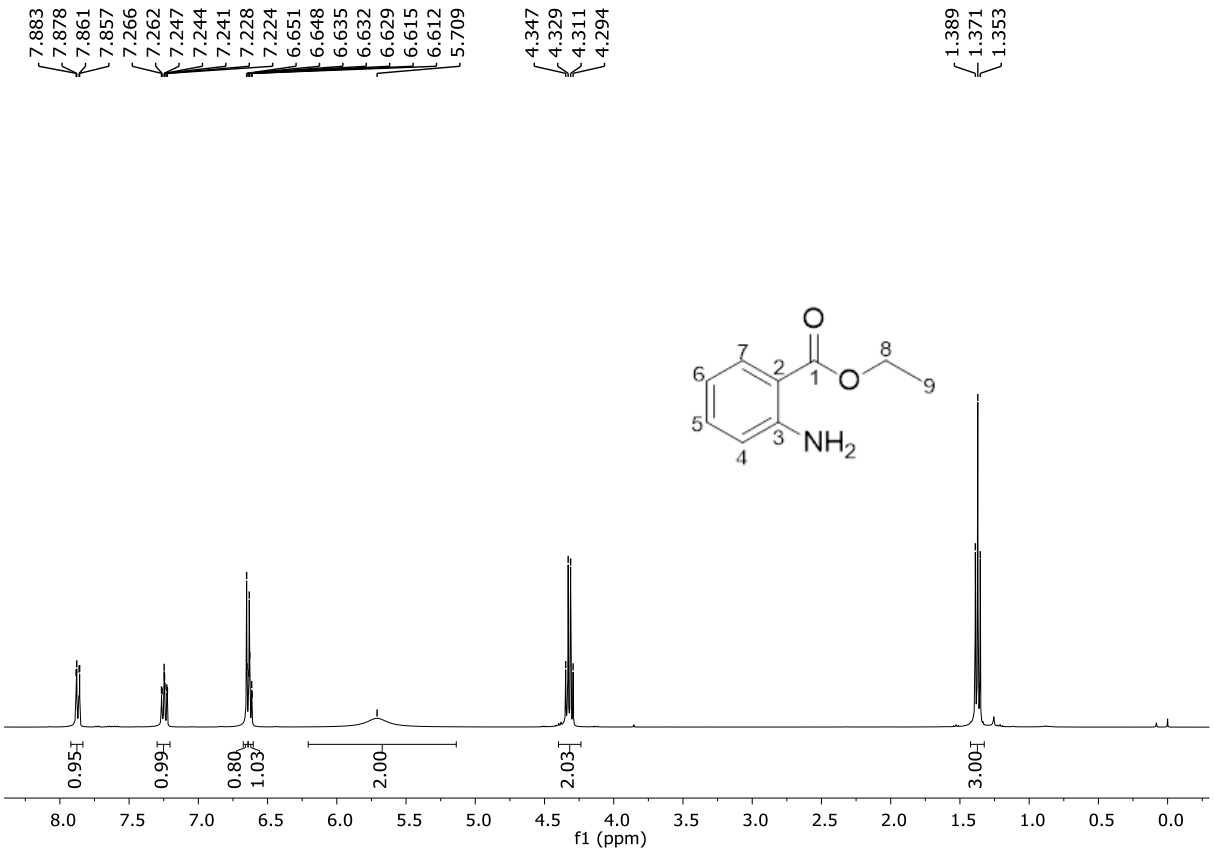


Figure S47. ^{1}H NMR for the compound 11a ($CDCl_3$, 400 MHz)

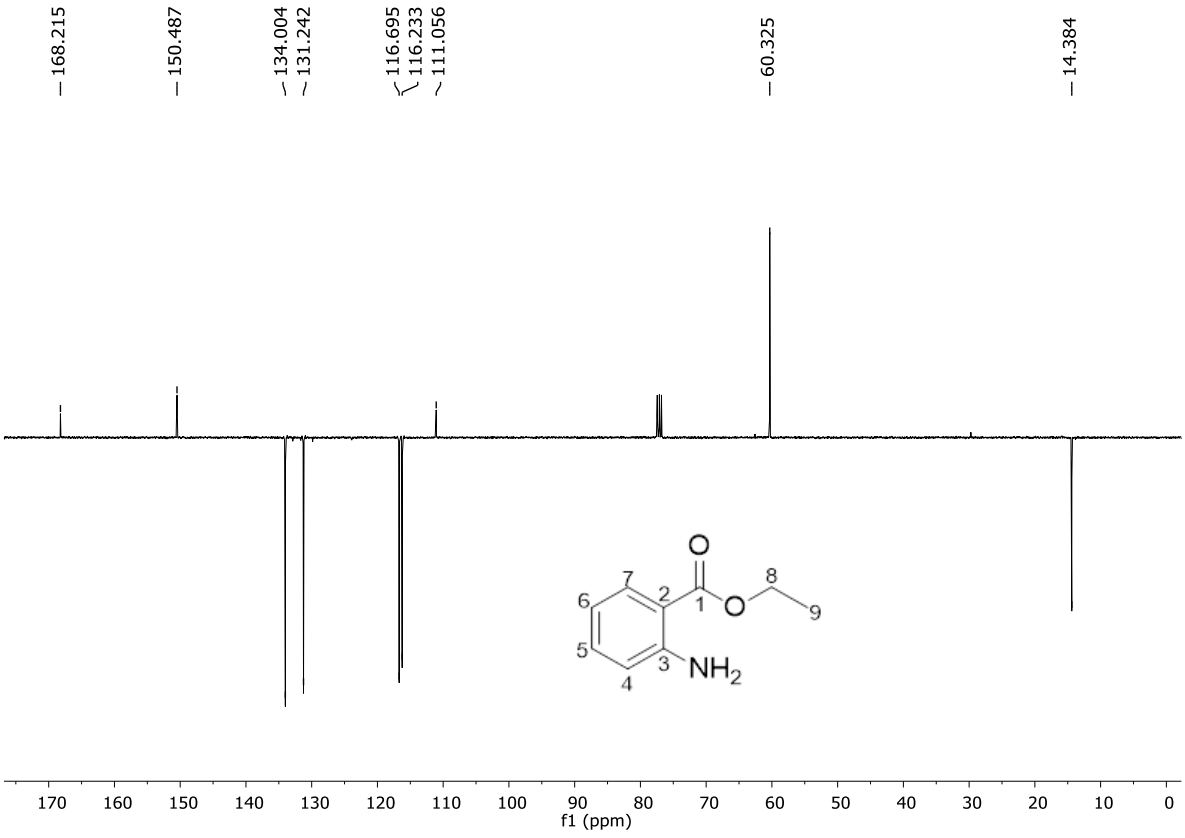


Figure S48. ^{1}H NMR for compound 11a ($CDCl_3$, 101 MHz)

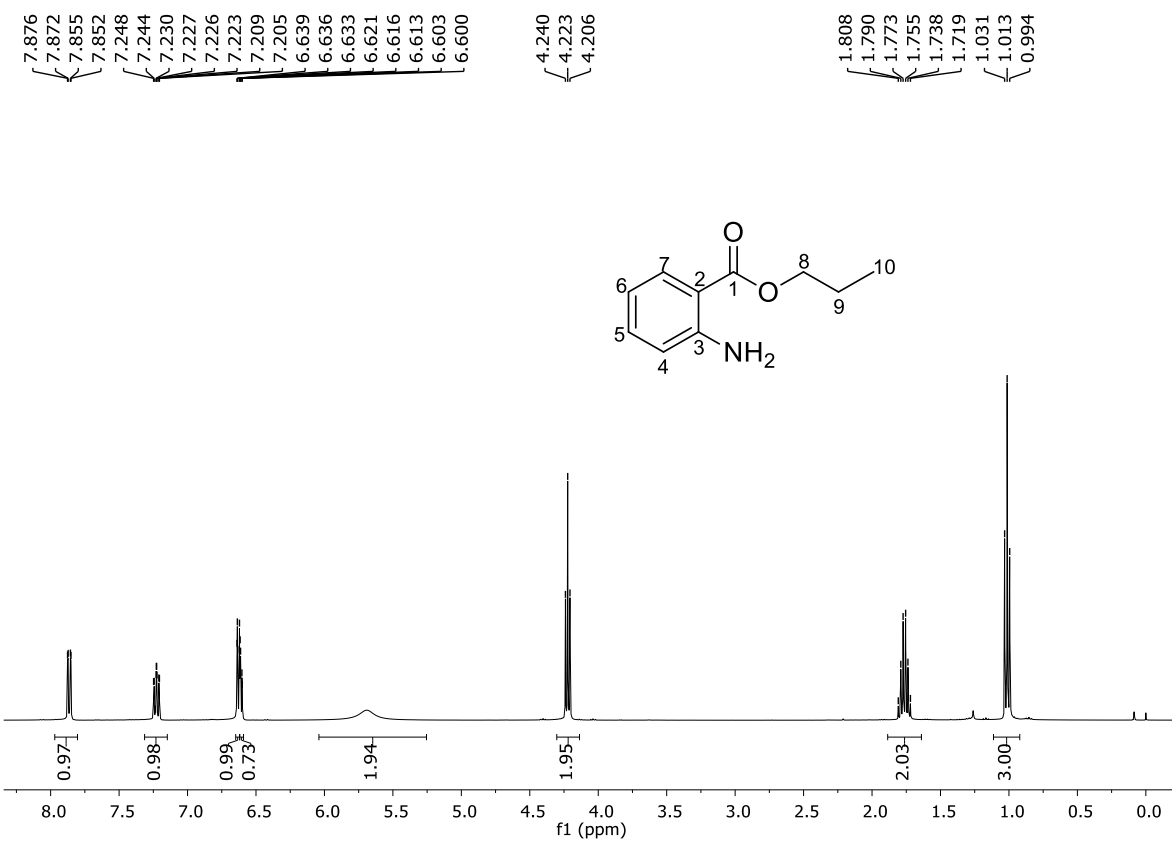


Figure S49. ^{1}NMR for compound 11b ($CDCl_3$, 400 MHz)

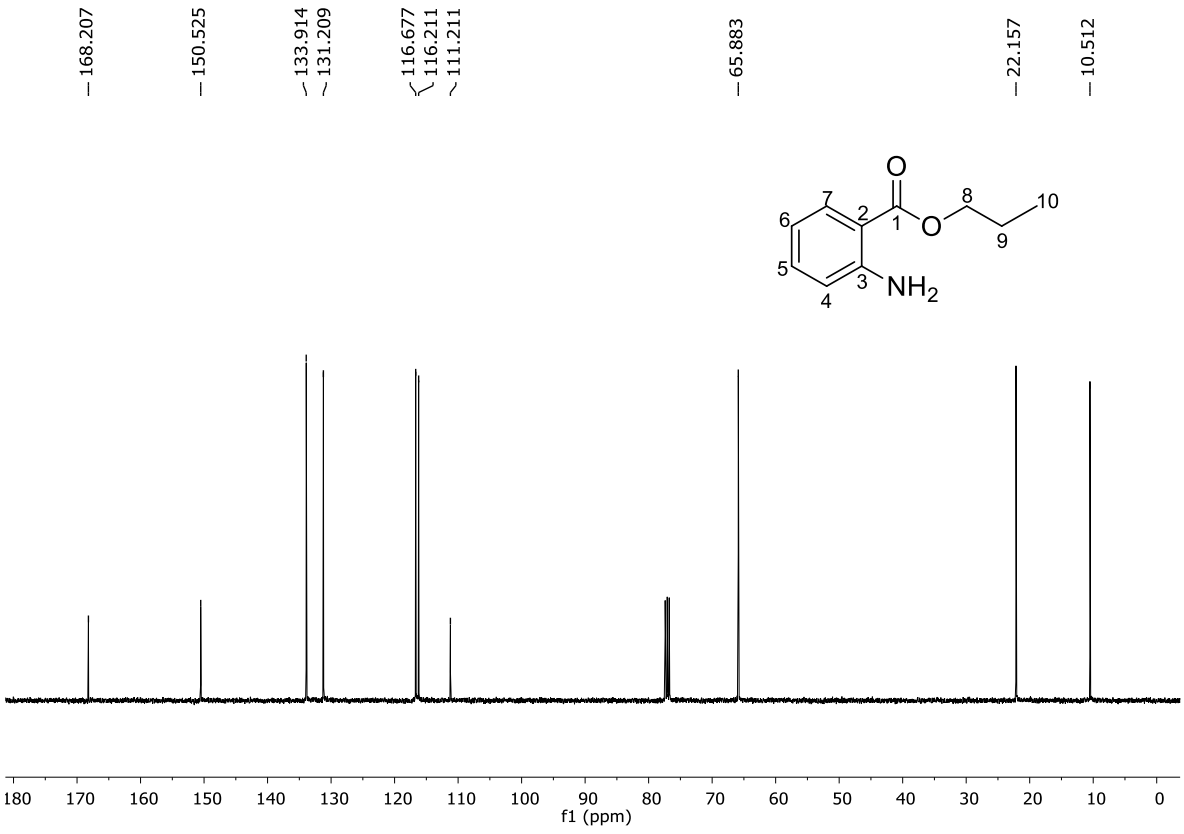


Figure S50. ^{13}C NMR for compound 11b ($CDCl_3$, 101 MHz)

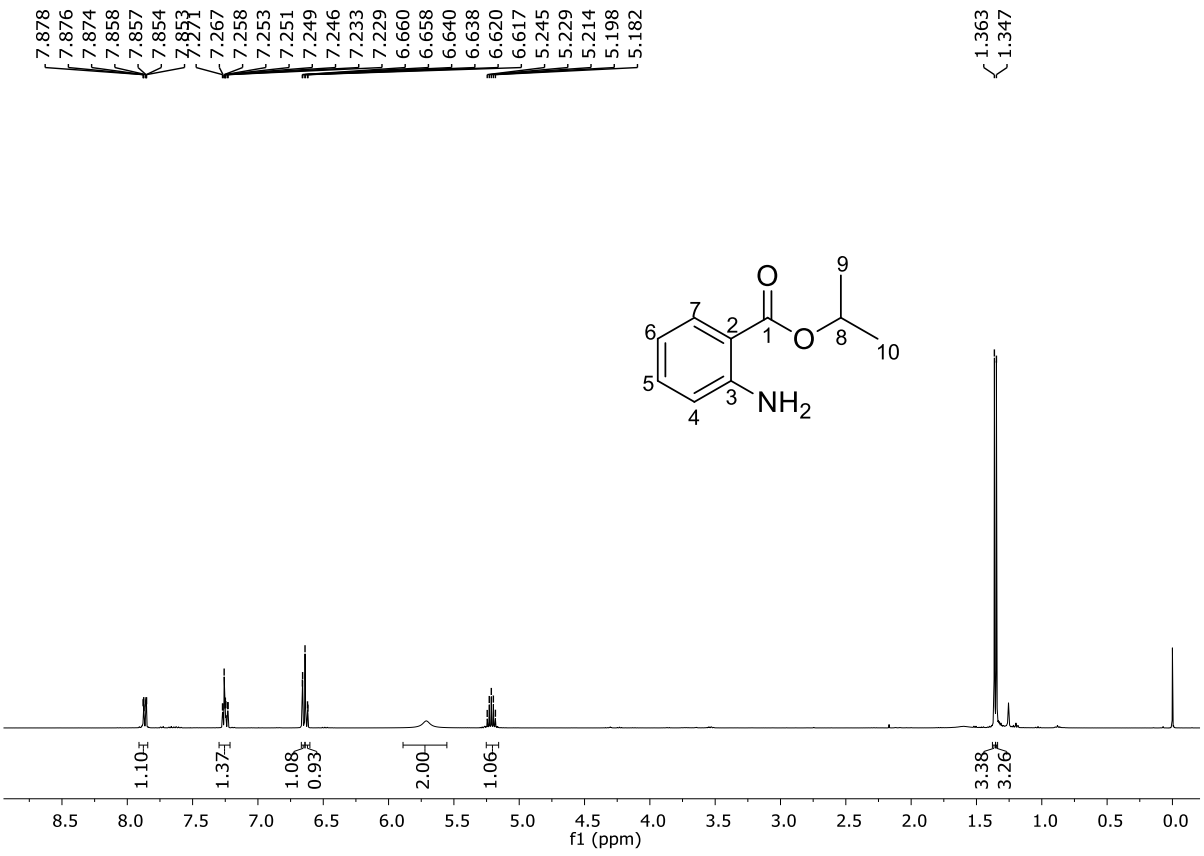


Figure S51. ^{1}NMR for compound 11c ($CDCl_3$, 400 MHz)

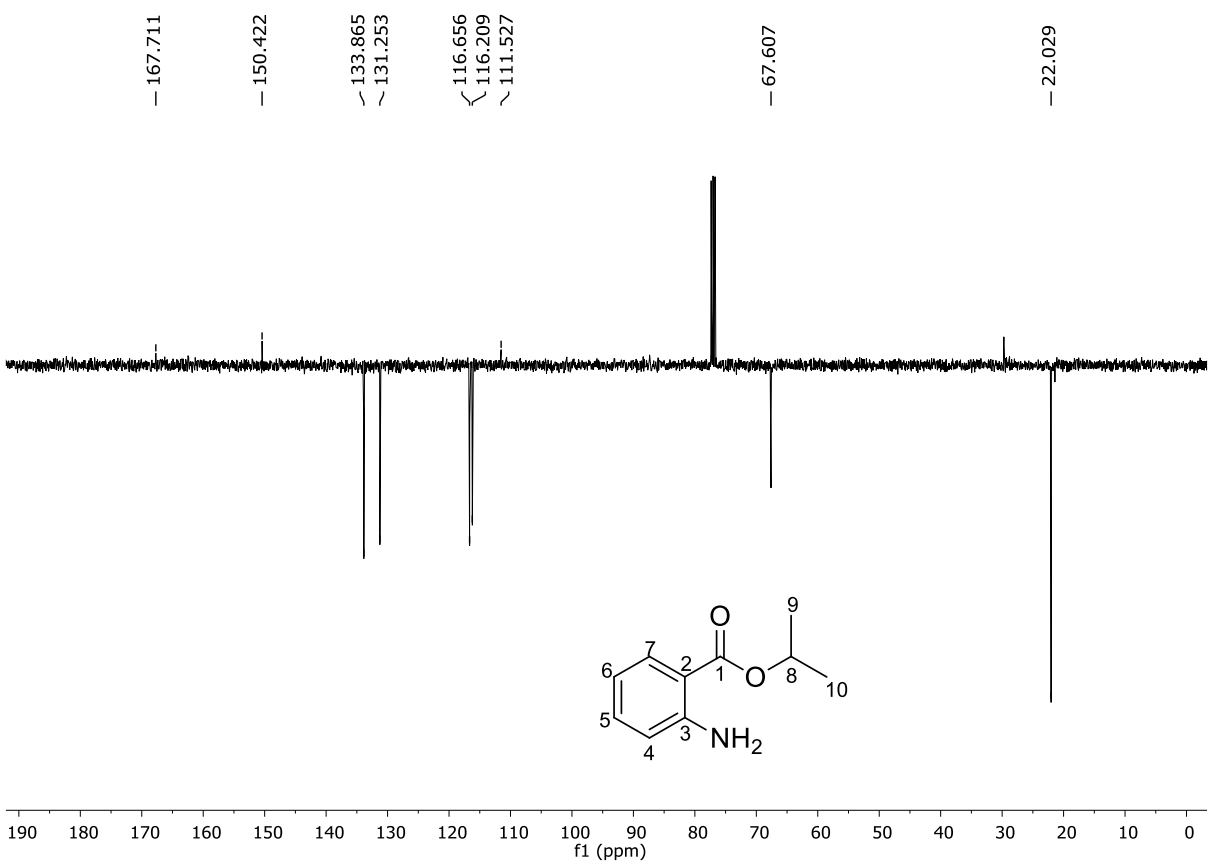


Figure S52. ^{13}C NMR APT for compound 11c ($CDCl_3$, 101 MHz)

CAPÍTULO 2. Synthesis and Anticancer activities and preliminary structure-activity relationships of Limonene and Perillyl alcohol Derivatives

1. Introduction

Cancer is responsible for millions of deaths worldwide each year, so it is highly important the necessity of studies concerning the mechanisms of action and new efficient treatments [1, 2]. Moreover, searching for novel molecules capable to treat cancer or engineering entirely new chemical entities such as small-molecules probes and drugs [3, 4] is one of the major concerns among the pharmaceutical and medicinal scientists, who investigate this topic.

Since the last decades, renewed interest of natural molecules as scaffolds and a significant number of drugs are in the market based on nature feedstock [5], demonstrating the capacities and innovation to discover new anticancer agents from this source.

Among these compounds, limonene (1), a small naturally occurring, monocyclic terpene is easily obtained and has significate importance against cancer, demonstrated in early works by Haag *et al.* in 1992 [6], and it is still studied extensively nowadays [7, 8, 9]. Limonene and its metabolites were also studied after oral administration in volunteers [10] demonstrating relevant activity in chemotherapy [11]. Among its metabolites, one of the most promising, perillyl alcohol (POH) is also found in nature [12] and like limonene, presented relevant anticancer activity [13, 14].

The use of Synthetic Organic Chemistry is a powerful tool to improve the activity, overcome limitations and give new properties to parent molecules, and is as well a modern topic in drug discovery [15]. As both limonene and POH are compounds presenting pharmacological activities, a synthetically intervention that could increase their natural effects is beneficial. In regard of limonene and POH, the early works by Crowell *et al.* in 1994 [16] and Eummer *et al.* in 1999 [17] were the first examples of the use of synthesis using these scaffolds in the last century.

Since the 2000s, some works explored the synthesis of limonene and perillyl alcohol derivatives as potential anticancer agents. Herein, for the first time, this information is reviewed by a synthetic point of view. The data is summarized and reorganized by affinity and type of strategy used. These synthetic strategies were

applied to improve the natural antiproliferative effect of limonene and perillyl alcohol and to overcome possible problems related to blood–brain barrier permeation. Strategies and modifications in the isopropenyl group and cyclohexane ring will be discussed in the next sections in order to be used as a source for future works of medicinal chemists hoping to design new anticancer agents based in these terpenes.

1.1 Modification at isopropenyl group in limonene and perillyl alcohol

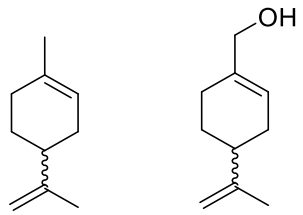


Figure 1. Limonene (**1**) and perillyl alcohol (**2**) chemical structures

The limonene (**1**) and perillyl alcohol (**2**) structures differ only by the presence of a methyl or a hydroxymethyl group (Fig. **1**). By similarity, these terpenes share two common chemical modification sites: the isopropenyl chain and the double bond present in the cyclohexane ring. The double bond region in the cyclohexane ring is less explored in Medicinal Chemistry and some works present only the synthesis of some limonene derivatives [18, 19]. The regioselectivity of these molecular regions will affect the choice of which groups will be inserted and can limit the molecular diversity achieved using these terpenes. The first modification addressed at the limonene and perillyl alcohol at the isopropenyl site is the amine modification [20].

1.2 Amine modification

The main reason to insert amines in the structure of limonene and perillyl alcohol is to increase the natural antiproliferative effect of these terpenes and the polarity, due to the low blood-brain barrier (BBB) penetration of these compounds, needing higher concentration to be effective [21, 22]. An increase in these parameters could lead to derivatives being active at lower concentrations and able to cross the BBB.

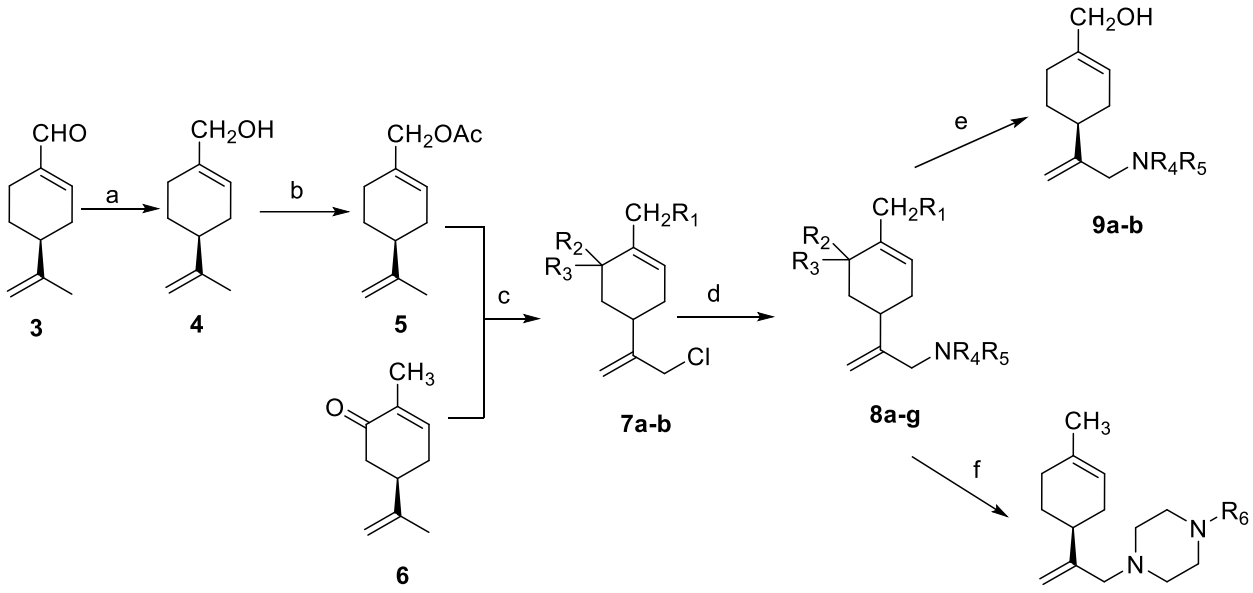
Chen *et al.* in 2006 started the synthesis by modifying L-carvone **6** with *tert*-butyl hypochlorite to obtain intermediary **7b** with 76% yield (Scheme **1**). With **7b,** the *N*-arylpiperazine derivatives were inserted with potassium carbonate and ethanol to obtain the series **8c-g** with the yields in the range of 61-77%. To obtain the limonene derivatives, the carbonyl group present in **8c-g** was reduced by Wolff-Kishner reaction with hydrazine and potassium hydroxide, to afford the L-limonene series **9c-g.** In this last step of carbonyl reduction, the yields were 10-15% [20].

Hui *et al.* in 2014 later revisited the same modification in the isopropenyl portion of perillyl alcohol to obtain the amino L-POH derivatives **9a-b** (Scheme **1**) [21]. In this work, Hui started with the L-perillylaldehyde **3**, which was reduced with sodium borohydride to the L-POH **4** with an 86.7% yield. To prevent side reactions the hydroxyl group in **4** was protected by acetylation with acetic anhydride (95.4% yield), and converted to the chlorine intermediary **7a** with 85.4% yield, with sodium hypochlorite. The selected amines in this series reacted with **7a** to afford the protected derivatives **8a-b**, in the range of 40-54% yield. These protected derivatives with acetate, were deprotected with sodium hydroxide in water to achieve the final amino L-POH compounds **9a-b**.

In regards of the synthetic route, both limonene and perillyl alcohol in these studies share some similarities. Both authors did not started directly with limonene or perillyl alcohol, needing to adapt the route to obtain these moieties. Another similarity is that the route share a common step, which consists the use of hypochlorite to obtain the chlorine derivative **7a** or **7b** necessary to react with the amines. The method used by Hui *et al.*, takes only 0.5 h to complete with a high yield of 85.7%, in contrast with Chen *et al.*, that required 3 h to afford the chlorine derivative in 76.0% yield. The final steps differ where the limonene derivatives **9c-g** were obtained by Wolff-Kishner reaction and the L-POH derivatives **9a-b** were achieved by deprotection of the hydroxyl group. In general, the introduction of the amino group in these cases was carried out regioselectively in the isopropenyl portion, proving that the amine modification at this region is affordable.

In regards of the structure activity relationships (SAR) observed to these series of derivatives, both were tested for various cancer cell lines being active in specific cell

lines and are more active than limonene and POH. In this review, we are going to focus in the most active derivatives.



7a and **8a -** R_1 = OAc, R_2 = R_3 = H, *R*-configuration

7b and **7b -** R_1 = H, R_2 = R_3 = C=O, *R*-configuration

Scheme 1. Synthesis of derivatives by amine modification. Conditions: (a) $NaBH_4$, EtOH, 0 °C to r.t., 3 h; b) Ac_2O, pyridine, r.t., 4 h; c) NaClO, AcOH, 0 °C, 0.5 h; d) R_1R_2NH, K_2CO_3, EtOH, reflux, 8–12 h; (e) NaOH, H_2O, reflux, 2 h (f) $NH_2NH_2.H_2O$ (85%) and KOH.

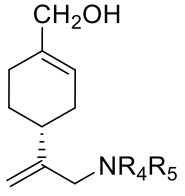
In the work of Chen *et al.*, the compounds presented activity against human prostate LNCaP cancer cells. The series of *N*-arylpiperazine derivatives of limonene (**9c-g)** were active against these cancer cells, with IG_{50} below 50 μM (Table **1**). The substituted aryl group with methoxide in the *ortho* position **9g (**20 μM) were t.he most active of the series, with a lower IG_{50} than his *para* counterpart **9f** (28 μM). Benzyl derivative **9e** presented activity of 24 μM, lower than **9f** suggesting that one carbon spacer could increase the activity. The remaining compounds **9c** and **9d** presents alkyl

groups in the *para* position (methyl and ethyl respectively), being the least active (38 and 50 μM). In regards of their mechanisms of action, these derivatives are not farnesyl transferase inhibitors but their antiproliferative effects are correlated with activation of ERK and induction of $p21^{waf1}$, proteins related in the processes of cell growth and proliferation.

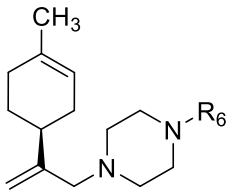
Hui *et al.* tested his derivatives in three other cell lines: A549 lung cancer, HT1080 fibrosarcome and A375-S2 melanoma. Besides introducing substituted *N*-arylpiperazines, other bulky amines such as piperidine were evaluated. The most promising derivatives **9a** and **9b** (Table **1**) presented IC_{50} of 59 μM and 73 μM for HT1080, being inactive in the other tested cell lines. Derivative **9a** is an *N*-arylpiperazine with a methoxide group in *para* position and **9b** has an amino pyridine substituent, suggesting that the introduction of heterocyclic amines could lead to active derivatives. Unfortunately for **9a** and **9b** no evaluation of the mechanism of action in the tested cells was performed.

In general, the introduction of amines at the isopropenyl site of limonene and POH increased the antiproliferative activity of and is a valid strategy to synthesize new derivatives.

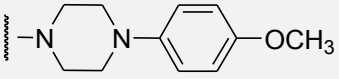
Table 1. Chemical structures, yields and activity of the most active compounds from the L-perillylalcohol (**9a-b**) and L-limonene (**9c-g**) series.

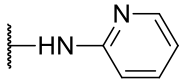
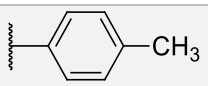
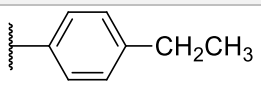
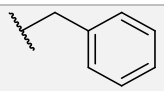
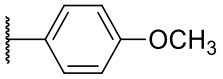
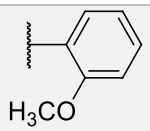


9a-b



9c-g

COMPOUND	NR_4R_5	R_6	YIELDS (%)	IC_{50} (μM)
9a		-	54.6	59[b]

9b		**-**	40.1	73[b]
9c	**-**		12.5	50[a]
9d	**-**		10.8	38[a]
9e	**-**		14.9	24[a]
9f	**-**		11.7	28[a]
9g	**-**		14.6	20[a]
L-limonene				>100 [ab]
L-Perillyl Alcohol				>1000 [ab]

a: LNCaP human prostate cells; **b**: HT1080 fibrosarcoma cells. Source: Chen *et al.* (2006) and Hui *et al.* 2014.

1.3 Urea and Thiosemicarbazone groups

Other possible modification is to synthesize urea and thiosemicarbazones at the isopropenyl site of limonene, in order to increase the antiproliferative activity, a strategy similar to amine modification.

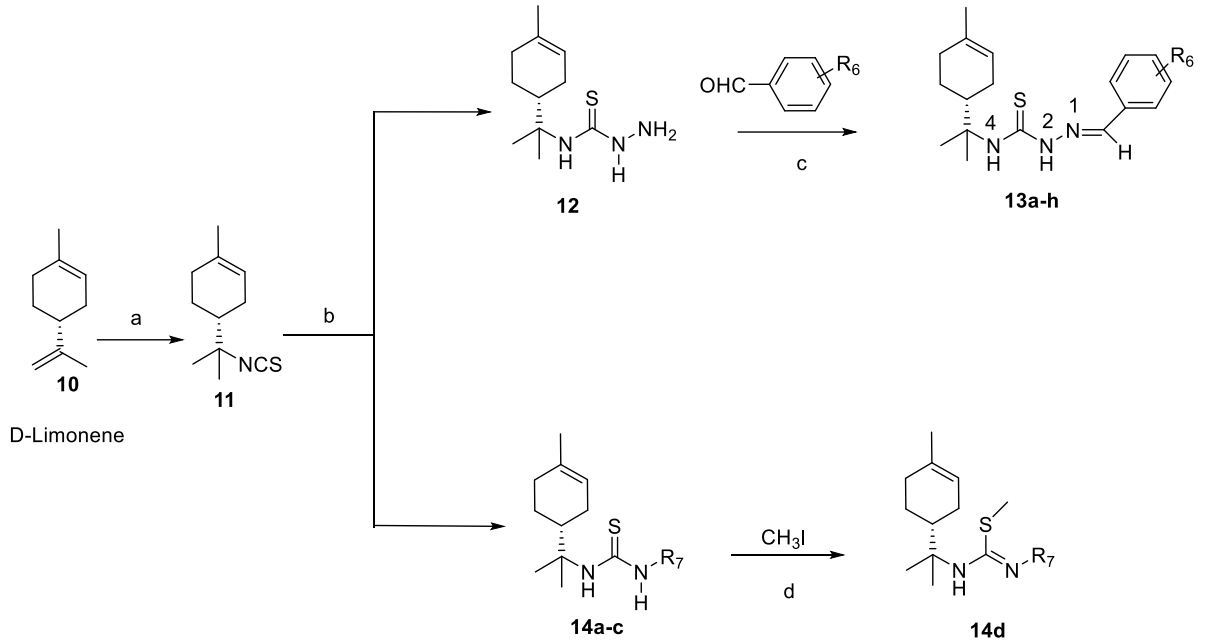
Figueiredo *et al.* in 2006 explored the synthesis of novel D-limonene derivatives attached with thiourea and S-methylthiourea moieties (series **14a-d**) (Table **2**) [22]. In this route, the first step was to convert D-limonene (**10**) with thiocyanic acid (HSCN) to the corresponding limonene isothiocyanate **11**[23]. With **11**, the next step was to react this intermediary with aromatic or aliphatic amines to afford the thioureas (**14a-c)** in a range of 30-80% yield. Higher yields were obtained with aliphatic amines (>70%) than aromatic amines (~30% e.g. **14a**). The S-methylthiourea (**14d**) could obtained by direct methylation of thioureas with methyl iodide in quantitative yields.

Vandresen *et al.* in 2014 presented a similar synthesis with substituted thiosemicarbazones with D-limonene (series **13a-h)** (Table **2**) [24]. This class is known for their promising anticancer activities [25]. In order to be effective the D-limonene moiety must be at N-4 position (Scheme **2**). In this route **11** is also obtained, and reacts with hydrazine monohydrochloride, sodium bicarbonate and water in ethanol reflux, to afford thiosemicarbazide **12** with 85% yield [26]**.** The derivatives **13a-h** were obtained by reaction of **12** with aldehydes in ethanol and HCl 10% in room temperature, with yields in the range of 75-95%.

The routes applied in both works are very similar, even including a common step that is the synthesis of limonene isothiocyanate **11**. This derivative is important because it is the base of both thioureas and thiosemicarbazones. To afford the thiosemicarbazones an additional step with aldehydes and thiosemicarbazide **12** is necessary. Remarkable to mention, the obtention of series **13a-h** is in mild conditions with fast reaction times (1-2h). In general both routes requires few steps with yields higher than 60%.

For structure activity relationships, both studies explored the antiproliferative activity of the derivatives **14a-d** and **13a**-**h** in diverse cancer cell lines, including melanoma UACC-62, breast MCF7 and lung NCI-460. No one of these studies presented information in regards of mechanisms of action of the most active derivatives.

The most active compounds in the study of Figueiredo *et al.* were the compounds with cyclohexyl **14b-c** and only one with phenyl substituent (**14a**) with the lower IG_{50} of 2.5-3.0 μM . These compounds were active in a variety of cell lines, with the exception of the N-methylpiperazine group (**14b**) that was very active for leukemia only (IG_{50} 3.0 μM). In addition, the methylation of the sulfur of the thiourea, presented only one active derivative with a cyclohexyl substituent **14d** in these series, with an excellent activity for leukemia (0.41 μM), as the N-methylpiperazine. In general, the introduction of thiourea and methylthiourea moiety in the structure of the D-limonene increased the activity and presented excellent results for specific cell lines.

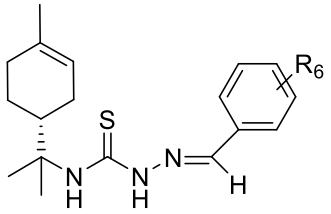


Scheme 2. Synthetic route for ureas and thiosemicarbazone derivatives. Reagents and Conditions: a) HSCN, CHCl3 rt. 48h; b) R_1R_2NH; (c) EtOH, HCl 10%, r.t. 1-2 h d) ICH_3, $CHCl_3$, 0ºC, 24h

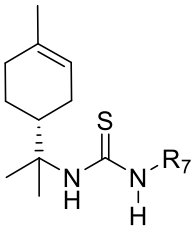
The introduction of the thiosemicarbazones **13a-h** in the work of Vandresen *et al.* with benzaldehyde derivatives in N-4 in the isopropenyl portion of limonene presented an excellent activity for various cancer cells (>1 μM). All derivatives **13a-h** showed remarkable antiproliferative activity (>0.04 μM) for PC3 cancer cell lines even being more active for this cell line than the reference drug paclitaxel (0.1 μM) [24]. The introduction of electron donating groups (methoxide, hydroxyl and methyl) in **13a, 13b** and **13c** in both *para* and *ortho* position was beneficial. The same is attested for **13e, 13f** and **13g** with halogens (Cl, F) in *ortho* and *para.* While **13a-h** presented pronounced activity for PC3, they presented promising activities for other cell lines, (e.g. **13h,** 0.1 μM, leukemia K-562) as exemplified in Table **2**. A brief SAR is presented in Figure **2.**

This work corroborates the findings by Li *et al.,* that instead of D-limonene studied the introduction of bulky groups at N-4 position of thiosemicarbazones ligands [27] with excellent results, specially one derivative with a 2-benzoylpyridine portion with IC_{50} of 0.002 μM for K562 leucocythemia cell line and 0.138 μM in the BEL7402 liver cancer cell line.

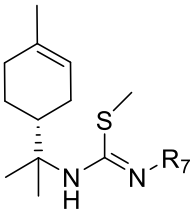
Table 2. Structure, yields and activity of the most active compounds against the three best cancer cell lines according Figueiredo *et al.* 2006 and Vandresen *et al.* 2014.



13a-h

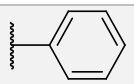
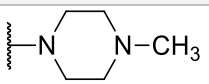
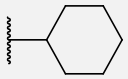
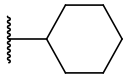


14a-c



14d

COMPOUND	R_6	R_7	YIELDS (%)	RANGE OF IG_{50} (μM)	CANCER CELL LINES
13a	4-CH_3	-	95	0.9-15.2	Breast MCF-7, Leukemia K-562, Prostate PC-3
13b	4-O-CH_3	-	90	0.04-7.9	Prostate PC-3, Ovarian OVCAR-3, Leukemia K-562
13c	2-OH	-	75	0.04-1.9	Lung NCI-H460, Prostate PC-3, Leukemia K-562
13d	4-$N(CH_3)_2$	-	89	0.04-26.8	Breast MCF-7, Leukemia K-562, Prostate PC-3
13e	4-F	-	80	0.04	Prostate PC-3
13f	2-Cl	-	94	0.05-15.1	Prostate PC-3, Ovarian OVCAR-3, Leukemia K-562
13g	4-Cl	-	91	0.05-7.8	Lung NCI-H460, Prostate PC-3, Leukemia K-562

13h	Furan	-	80	0.1-9.3	Breast MCF-7, Colon HT-29, Leukemia K-562
14a	-		30	2.5-15.6	Breast resistant NCI/ADR, Melanoma UACC-62, Prostate PC-3
14b	-		90	3.0	Leukemia K-562
14c	-		80	3.0-12.5	Ovarian OVCAR-3, Breast resistant NCI/ADR, Prostate PC-3
14d	-		QT.	0.41-39.1	Ovarian OVCAR-3, Leukemia K-562, Colon HT-29
D-Limonene	**-**	**-**	**-**	**>100**	**ALL**

Source: a: Figueiredo *et al.,* 2006 [22]; b: Vandresen *et al.* 2014. [24]

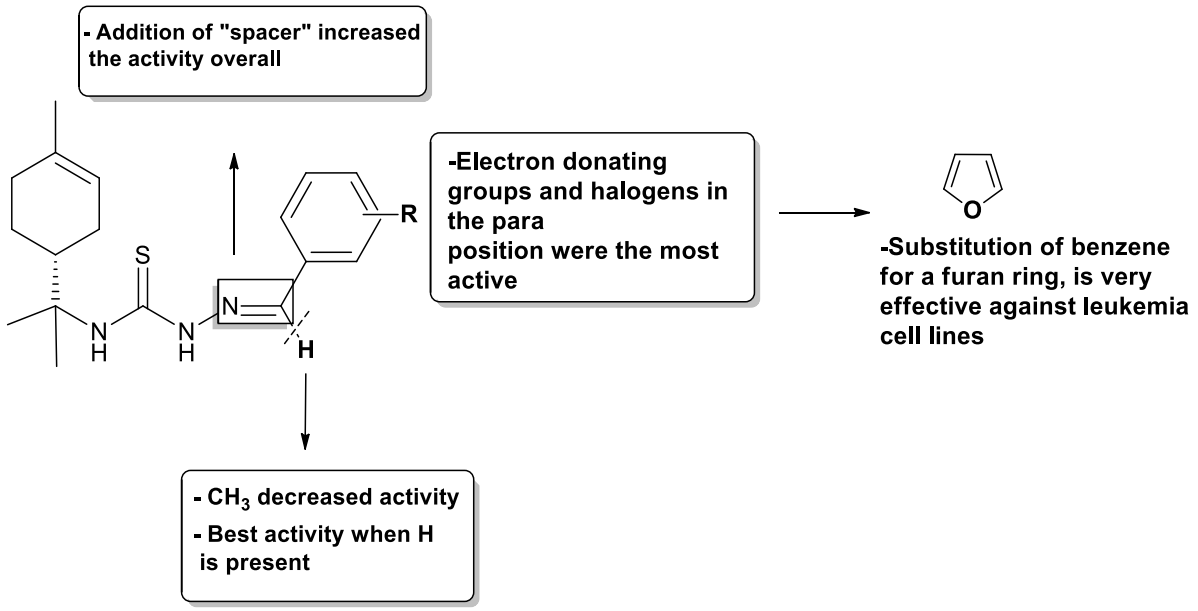


Figure 2. Structure Activity relationships of the thiosemicarbazones **13a-h** of the work of Vandresen *et al.*

Both of series of ureas and thiosemicarbazones derivatives has pronounced antiproliferative activities for specific cancer cell lines, being a valid group to introduce at the isopropenyl site that increased the natural anticancer effect of limonene. In addition, the thiosemicarbazones derivatives were all aromatic and heteroaromatic with variations of substituents indicating that heterocycles may be a good “toolbox” choice to begin modifications in limonene.

Both of thioureas and thiosemicarbazones are still explored nowadays and have a great potential toward lead compounds. One of these examples is compound **13e** with fluorine in the *para* position that years later in 2018, was explored in regards of its mechanism of action and safety profile related to antitumor activity against PC-3 adenocarcinoma cells. Derivative **13e** is capable to induce apoptosis via caspase-9 and increase p53 levels. In addition, **13e** is suggested to have low acute oral systemic toxicity [28].

2. Modifications at the hydroxyl group of POH

The presence of a hydroxyl group at POH offers a good opportunity to perform synthetic modifications such as molecular hybridization and conjugation with carbohydrates. Hybrid drugs and molecules have special attention nowadays in medicinal chemistry [29]. Since 2000, some authors explored POH derivatives modified at the hydroxyl group and this will be discussed in a chronological order and grouped in regard of structural similarity.

2.1 Fatty Acid, Carbohydrates and Phospholipids

2.1.1 Fatty Acids and Phospholipids conjugated with POH

One possible logical way is to take advantage of the hydroxyl portion of POH to synthesize esters or ethers. The reason is to increase the antiproliferative activity and to increase the hydrophilic content, as perillyl alcohol present limited solubility, similar to limonene, and can cause problems to cross the BBB.

Skouridou *et al.* proposed an esterification of perillyl alcohol with fatty acids, inspired by the work of Kodelia *et al.* with rutin esters in 1994 [30, 31]. The main reason as suggested by the author, was to increase the antiproliferative effect and to increase the hydrophobic content of POH. Yet, in this work, only the synthesis was evaluated. Instead of conventional methods, the esterification was carried out by an enzymatic method by one lipase isolated from *Candida antarctica* to afford the derivatives **19-21** (Scheme **3**). The reaction have different rates of conversion and is dependent of the solvent, water content and the substrate. The results showed that acyl donor, decanoic acid **16** presented higher rates of conversion when the water content is set to 0.11. According to the author, better conversion is observed when in general the water content is lower than 0.32. The solvent need to have a logP higher than 3.5 and for **16**, α-pinene was the most appropriate solvent. In addition, *Candida antarctica* lipase prefers D-POH over L-POH esterification, meaning that this reaction is enantioselective. The author also verified that the enantioselectivity is independent of the water content and is not conclusive about the effect of the solvent.

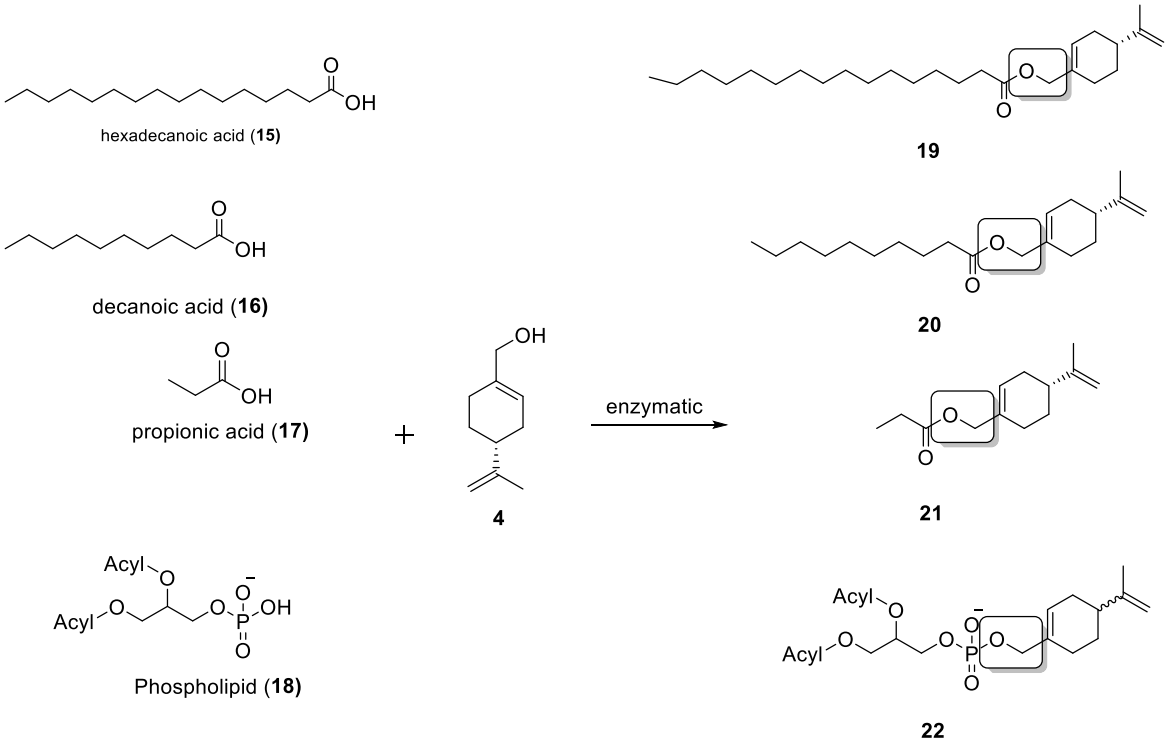
Lontrari *et al.*, used the same method with decanoic acid **16** to obtain **20,** and evaluated angiogenesis inhibitor activity of the POH and ester **20** [32].

Yamamoto *et al.,* also explored the same kind of approach in 2008 with phospholipase D (PLD), where perillyl alcohol was attached with a phospholipid **18**, based in a previous work where one derivative was phosphatidylated and presented higher antitumor activity than his previous counterpart [33, 34]. The author also suggested that phosphatidylated compounds are excellent candidates for drugs or fine chemicals. In this work, the PLD is mediated of phosphatidylcholine from soy bean (SoyPC) based in a previous study conducted by the author. The reaction conditions to obtain **22**, uses 50 µmol of soyPC, 2 mmol of POH, 1.6 U PLD and 0.8 ml of 0.2 M sodium acetate buffer, with pH settled to 5.6 (Scheme **3**). The reaction was conducted at 37ºC for 24 h. The final yield of **22** is 79%.

The main idea behind these studies was to provide a fast and reliable one-step synthesis of these derivatives by enzymes and evaluate these derivatives in cancer lines, or in the case of POH ester **20**, evaluated its potential as angiogenesis inhibitor.

Loutrari *et al.* in 2004 verified that POH and not **20** was capable to interfere in angiogenesis. Solely, POH was capable to modulate two angiogenic regulators:

vascular endothelial growth factor and angiopoietin 2, indicating that may suppress neovascularization in tumors and stimulate the regression of blood vessels. and induced apoptosis of endothelial cells. These data indicated the importance to preserve the hydroxyl portion of POH for angiogenesis inhibition.



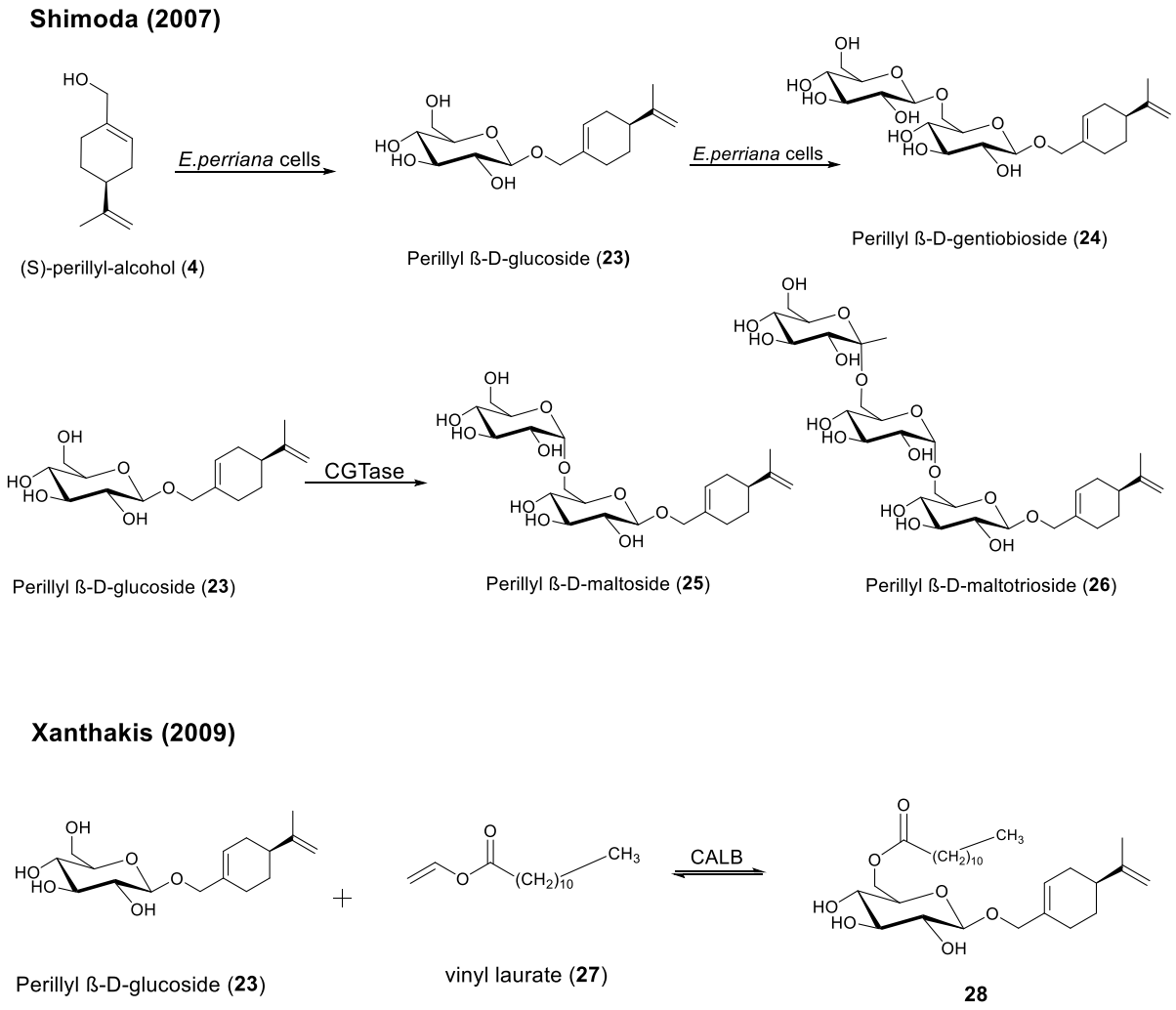
Scheme 3. Esterification of perillyl alcohol and Phospholipid conjugation with *Candida Antarctica* lipase (**19-21**) and phospholipase D (**22**)

Yamamoto *et al.* [33] has conjugated POH with phospholipids. This novel derivative **22** retained the activity at 100 μM in prostate PC-3 and leukemia HL-60 cell lines (WST-1 assay for cell proliferation) and modification of the hydroxyl group increased activity in comparison with POH (400 μM). No mechanism of action is studied or suggested by the author.

In these works, it was shown that the conjugates with fatty acids and phospholipids could be active and possibly motivates other researchers to make new modifications in order to find new derivatives with greater potential.

2.1.2 Carbohydrates

New highlights in this field were made when fatty acids were substituted by sugars to obtain POH glycosides. Fujita *et al.* [34] explored the activity of these glycosides against aldose reductase in 1995, but only in 2007, Shimoda *et al.* [35] presented a biocatalytic glycosylation of POH with unnatural glycosides by plant cell culture of *E. perriana*. The major products from these reactions were the unnatural perillyl- ß-gentiobioside with a yield as high as 60% and perillyl- ß-glucoside with a low yield of 4%. From this latest derivative, two novel unnatural glycosides (perillyl ß-D-maltoside and perillyl ß-D-maltotrioside) were obtained with respective yields of 27% and 7%. Inspired by these molecules, Xanthakis *et al.* [36] in 2009 engineered a POH glycoside by enzymatic synthesis studying the influence of various parameters in order to get a maximum conversion of perillyl glucoside from POH. In addition, a POH glycoside ester with vinyl laurate was studied by using the enzyme lipase B from *C. Antartica* (CALB). This derivative was obtained directly by the POH glucoside with a final yield of 83,9% with maximum conversion. As Xanthakis improved the synthesis in comparison with Shimoda, the acylation of the perillyl- ß-glucoside to give the laurate ester was novel at time. The main reason of the author, besides present an efficient enzymatic synthesis, was to compare the antiproliferative activity of the POH with the glucoside and laurate glucoside which present an increase in the hydrophobic content. The molecules were tested in the concentrations of 0.25 mM and 0.50 mM with LLC cells as the vehicle. The most active compound was POH, while the glycoside was almost inactive. Modification of the sugar portion by acetylation increased the antiproliferative activity in Lewis lung carcinoma cells in counterpart with the original perillyl- ß-glucoside only. While the glycosylation did not increase the activity higher as of POH, a modification of the sugar may be beneficial and a factor that can increase the outcome of these molecules. All products are demonstrated in Scheme **4**.

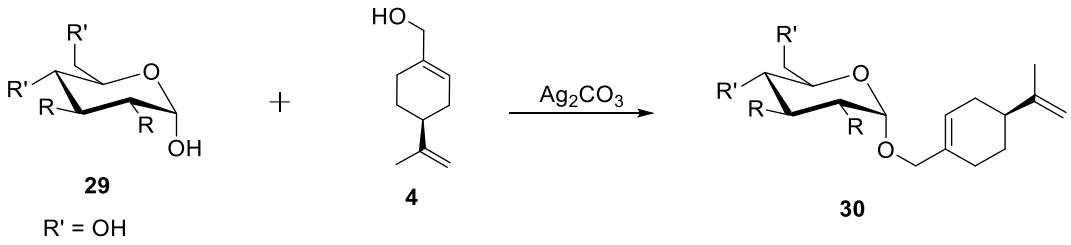


Scheme 4. Synthetic modifications using enzymatic synthesis to obtain perillyl-carbohydrates hybrids.

All these results indicated conclusions in regards of modification: while the solely modification of POH with a glucoside was not beneficial, maybe derivatives with varied substituents in portions of the sugar can produce molecules that are more potent than POH itself. This idea culminates in 2014 with Nandurkar [37] tested Neoglycorandomization for chemoselectivity and differential glycosylation for POH glycosides in order to increase the antiproliferative effect. Also, the use of a Huisgen 1, 3-dipolar cycloaddition catalyzed by Cu(I) to produce triazole, was novel at that time

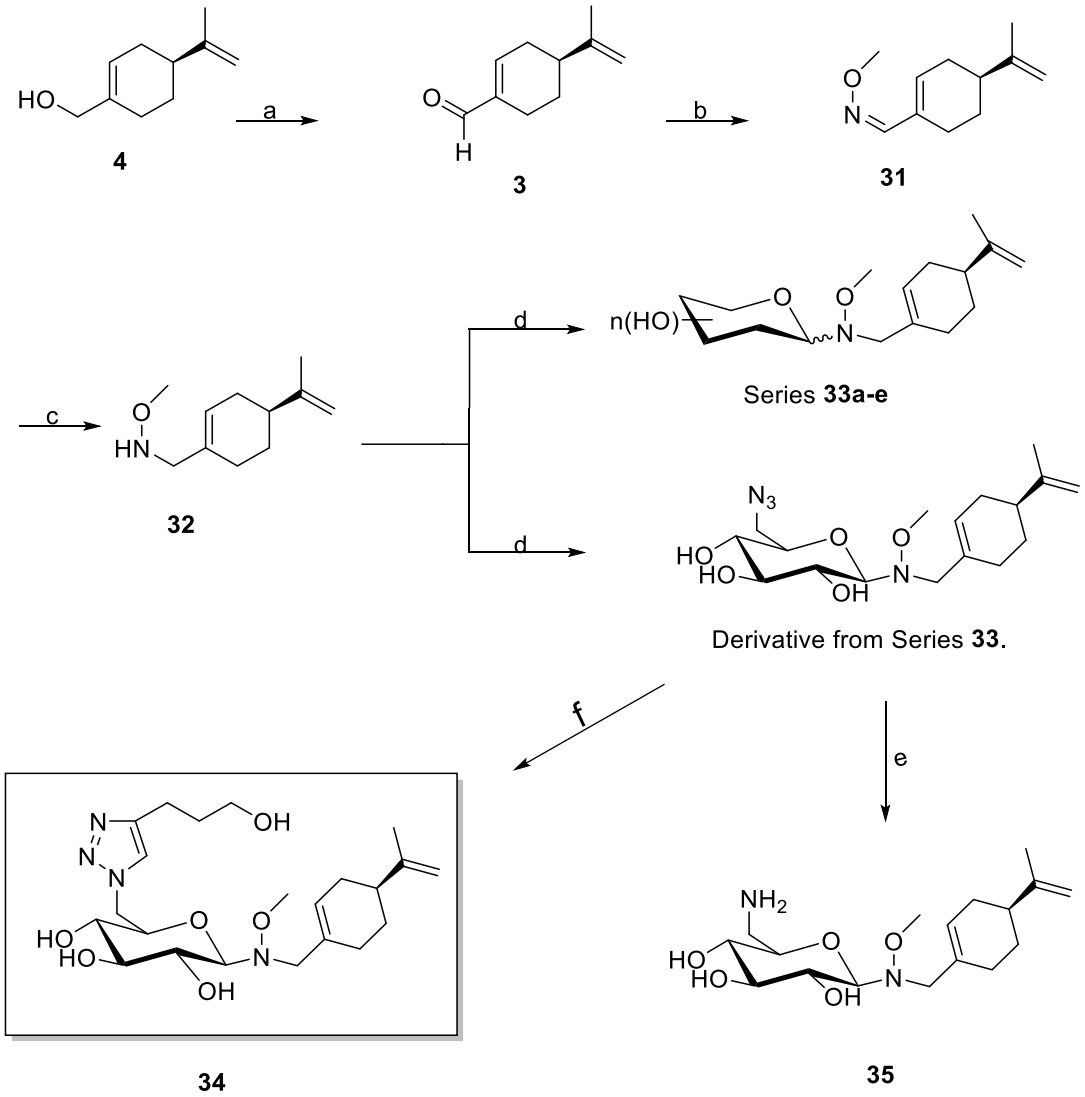
for this scaffold and compatible.

Synthetically, before Nandurkar, only the work of Fujita in 1995 [38] approached the glycosylation of POH by conventional methods. Koenigs–Knorr reaction with bromoacetoglucose was applied for this work (Scheme **5**) and is one of the oldest and reliable methods to synthesize glycosides.



Scheme 5. Koenigs-Knorr reaction applied in the work of Fujita (1995), to synthesize unnatural glycosides

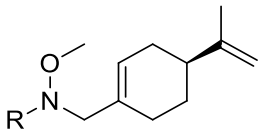
Even though is reliable, for industry scales, the other author's explored alternatives, such as enzymatic synthesis in the previous works. These syntheses are useful for obtain one direct product, but a more robust and specific method for functionalization is used such as conventional synthesis, as presented by Nandurkar [37]. The conversion of POH to perillylaldehyde is the first step (Scheme **6**). This is done by IBX and acetic acid with high yield of 88%. Later, the aldehyde is converted to oxime with methoxylamine hydrochloride with a high yield of 84%. In both of these steps, room temperature was applied. Oxime was reduced with $NaCNBH_3$. With this intermediary, the sugars can be attached. The finals are largely variable, from 20% to 77%. Novel at the time was the inclusion of azide group in the structure of the sugars with POH was a smart and modern alternative for the perillyl scaffold.



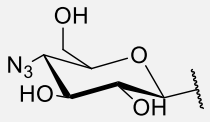
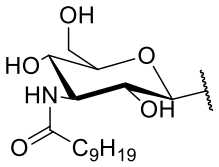
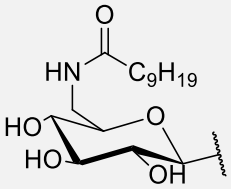
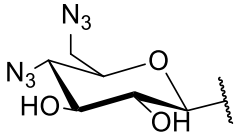
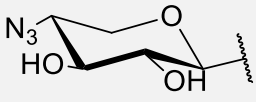
Scheme 6. Conversion of POH to Oxime. Reagents and conditions: (a) IBX, HOAc, acetonitrile (88%); (b) MeONH3Cl, Et3N, EtOH (84%); (c) NaCNBH3, HOAc, MeOH (50%); (d) reducing sugar, MeOH:HOAc (5:1), 40 °C (23%−77%) ; (e) PMe_3 in THF, overnight, (46%); (f) 4- pentyn-1-ol, CuI, Et3N, ACN, overnight, (82%)

This azide derivative from series **33** was later converted to triazol (**34**) by Huisgen 1, 3-dipolar cycloaddition catalyzed by Cu(I) or reduced to amines (**35**). This kind of approach increase the scopes of the study, and is possible to study the effects of differents substituents and positions of the azidosugars. Combined with Organic Synthesis and anterior evidence, this study was an enormous success. A library of 34 new derivatives with mild reactions conditions, and the most potent POH glycoside (still to date) were synthesized. The most active compounds are presented in the Table **3.**

Table 3. Most active compounds of the work of Nandurkar, with hybrids of perillyl alcohol and glucosides.



33a-e

COMPOUND	R	YIELDS (%)	IG_{50} OR IC_{50} (μM)
33a		73	4a; 22b
33b		64	14a; 19b
33c		62	14a; 30b
33d		38	44a; 57b
33e		40	76a; 170b
Perillyl Alcohol			**>100a,b**

A = in A549 cell lines, b = in PC3 cell lines

Manipulating the positions of the glycoside with the azides Nandurkar [37] synthesized the most potent perillyl glycoside derivative with the azide in position C4' in the sugar, with activity of 4 μM for A549 cell lines and 22 μM for PC3 cell lines. In comparison with POH that presented activity of 350 μM for A549 and 380 μM for PC3, the activity had increased greatly. The effect was not addictive when two azides were present as in compound **33d**. Other effective functional group was amide groupss with long hydrophobic chains as evidenced by **33b** and **33c**. Also, these compounds were more sensitive to A549 cell lines instead of PC3. To date, the derivative **33a** is the most potent perillyl glycoside obtained and could be later used as a lead. In addition, all of these works used as starting material the *S* configuration of POH, while the isomer *R* was almost unexplored, opening the field for new synthesis involving carbohydrates and perillyl alcohol.

2.1 Hybrids with existing drugs

Other possible path to hydroxyl modifications is to attach POH to existing drugs . Fonseca *et al.* [39, 40] demonstrated the potential of intranasal use of perillyl alcohol in the survival of patients of recurrent gliomas and glioblastoma (POH showed survival of 5.9 months compared with untreated group was 2.3 months).

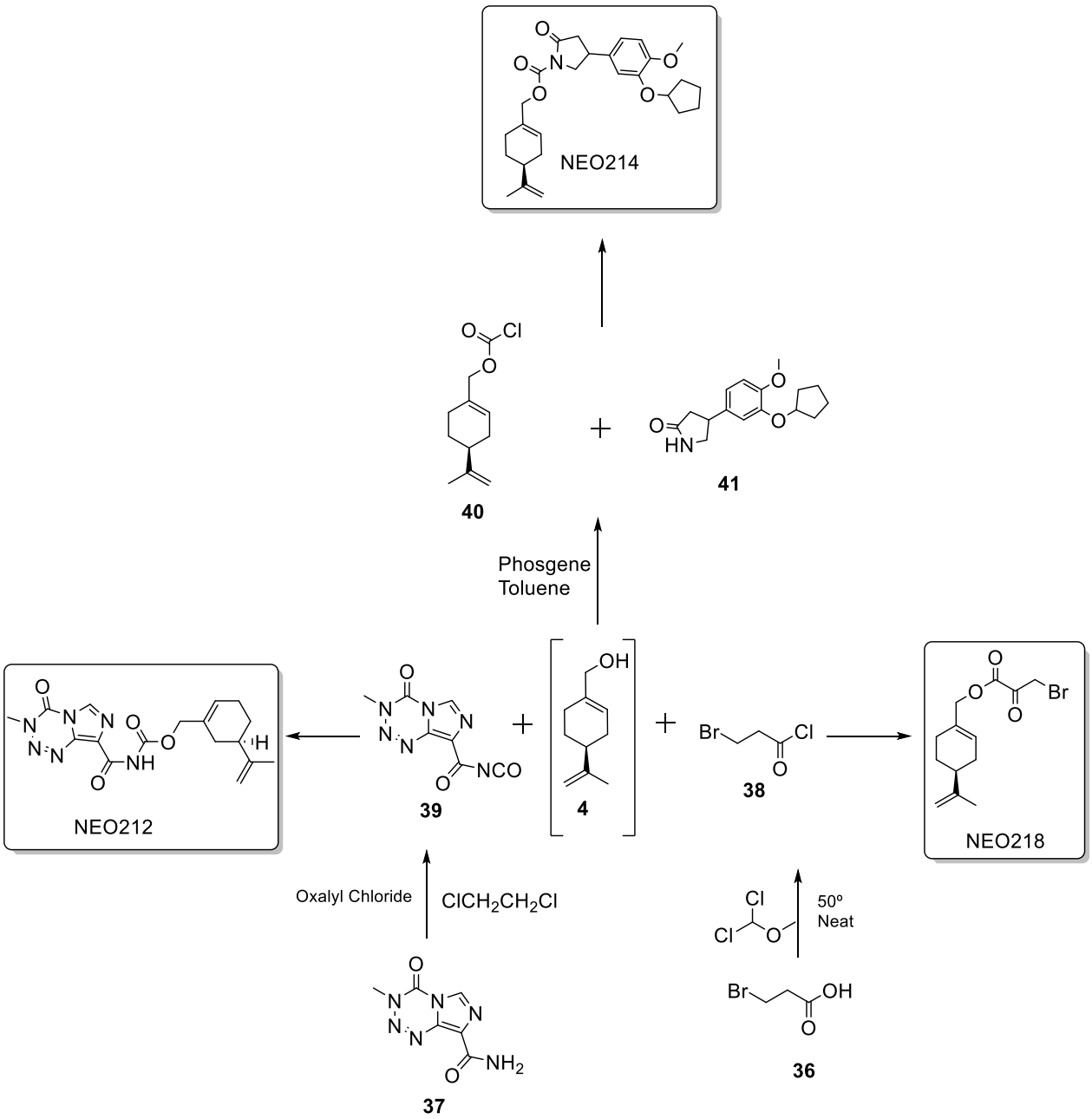
Cho corroborates these findings when perillyl alcohol showed activity against Temozolomide resistant gliomas, when administered intranasal [41]. With this in mind, Chen proposed a more direct and functionalizing strategy in 2014 [38]. Chen explored the perillyl alcohol and TMZ **37** (temozolomide) conjugate, in order to evaluate if this hybrid can improve antiproliferative activity and also improved the effectiveness of TMZ in regards of brain metastases of breast cancer, a problem that TMZ presents, even if can cross the blood brain barrier. In addition, the treatment has a high rate of death of the patients, with a resistance for some cell lines for TMZ. Therefore, it is important to find a new drug that can overcome these problems and increase surviving. The hybrid (named as NEO212) was synthesized and presented good activities of 500 mM in MM cancer cell lines. This opened a new field for exploration: functionalization of existing drugs with POH is a valid strategy.

Chen also proposed two more studies and hybrids with actual anticancer drugs: 3-bromopyruvate in 2017 [42] and Rolipram in 2018 [43]. These hybrids named NEO218 and NEO214, respectively. Currently a prototype with valproic acid and POH was in production [44].

Synthetically, two strategies were described in patents and showed in Scheme **7**. First modification of POH was the conversion of the hydroxyl group to acyl chloride with phosgene and toluene is a possibility, increasing the range of reactions that used to obtain hybrids. Chen used this strategy in the conjugate with Rolipram **41**, as the same present a pyrrolidin-2-one where the perillyl chloroformate can be attached via a carbamate bridge [45]. In the other studies (NEO212 and NEO218), the other strategy is to modify the existing drug [46, 47]. This is largely dependent if the drugs present groups that are passible of modification. TMZ have an amide present that can converted to carbamate, where POH can insert, by the hydroxyl portion. The use of a carbamate bridge as described by the author was successful in this case, as in the case of Rolipram. For the 3-BP **36**, modification is made directly in carboxylic acid group of the original drug to allow the insertion of POH. These strategies do not involve great modifications in the core structure of the original drug to preserve the core structure and activity, but groups that can suffer interconversion with minimum intervention in the overall structure are preferable. This can be increasing the difficulty of synthesis if the original drug does not present any group that can be modified or not compatible with the hydroxyl group of POH. In these cases, both the drug and POH were submitted to modifications in their parent structure in order to create the hybrid. In addition, the concept of attaching a drug was expanded recently when three bioactive molecules were attached together and assayed against melanoma cancer. This conjugate is a derivative of NEO212 plus linoleic acid, named NEO412 [48].

In regards of activity, these hybrids were a success in most of the cases. NEO212 was more active than their original counterparts tested alone, with a different mechanism of action of TMZ: presents the capacity of alkylate DNA and may induce endoplasmic reticulum stress and autophagy inhibition as new functionalization, absent in TMZ. The range of activity was effective, with IC_{50} of 8 μM for U251 cell lines being almost two times more effective than the reference drug TMZ. The most sensitive cell line was LN229 with IC_{50} of 5 μM, again, more effective than TMZ with IC_{50} of 10 μM. In addition, the higher IC_{50} were for the cells resistant to TMZ, indicating that this

hybrid can overcome the resistance of this drug, and in conjunct of its new functions, possibly be used in therapeutics against glioblastoma multiform. NEO212 was explored at the same year of its first publication expanding the range of TMZ- resistant gliomas [49], and glioma stem cells [50-52]. In addition, other types of cancer were explored [53-56].



Scheme 7. Synthesis of the Hybrids with existing drugs

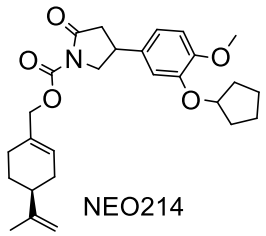
Besides NEO12, NEO218 the next explored hybrid with 3-bromopiruvate, an alkylating agent studied in the treatment of some tumors [57]. The hybrid presented similar activity with their individual counterparts and the same mechanism of action inducting necrosis of the cells. It is suggested however, that NEO218, may overcome some limitations in the original mechanism of action, as 3-BP needs a high level of MCT-1 in cells to work properly (with activities high in the range of 150-300 μM, for cancel lines with low levels of MCT-1). The hybrid, NEO218 is independent of the MCT-1 levels with an IC_{50} of 15-25 μM in ten varied cell lines, indicating a possible new mechanism of action of these hybrids. Also, as elucidated by the author 3-BP presents a rapid and increase resistance by the cell defense, while NEO218 may overcome a crucial mechanism of defense of the tested cells. Yet, more study is needed to understand the potential of NEO2018 [58].

The more recent hybrid NEO214 [62], however, was designed in order to increase activity against multiple myeloma (MM) and to overcome the dose-limiting GI toxicity when POH is administered orally (these weakness is not observed when POH is applied via intranasal delivery [59]). The activity of NEO214 was around 50 μM for the all five MM tested cells and presented sensitivity for cells resistant to doxorubicin, similar to what is observed with NEO212. The hybrid presented better activity in at least one-degree higher when compared to his separate members and, promote ER stress as important mechanism of action. While NEO214 was capable, it presented a great synergistic effect with bortezomib, the major drug used in the treatment of MM, with a high-pronounced effect. In 2019, NEO214 was investigated for glioblastoma multiforme, and presented activity for TMZ resistant cell lines [60]. It also induced apoptosis by ER Stress and Death Receptor 5/TRAIL pathway. Similar to the previous hybrids designed, the activity is increased (40 μM) when compared to its ineffective counterparts. As it safe to administer NEO214 for higher doses of 400 mg/kg this hybrid is still investigated as a possible anticancer agent.

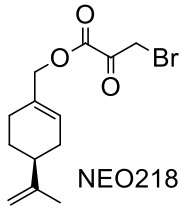
These three studies indicating a new potential way to use perillyl alcohol: the use of the same to produce hybrid molecules attached by the hydroxyl group by Carbamate Bridge or ester (as NEO218) with satisfactory results for glioblastoma multiforme and multiple myeloma. All information related is grouped in Table **4.** These

hybrids presented increased activity in comparison of the drugs or perillyl alcohol itself, triggered new mechanisms of action, and overcome some limitations that the original drugs present, such as resistant to some cell lines and high doses. This is verified when comparing NEO212 and NEO214, very active for TMZ resistant cell lines, with the last one being active for MM also. While NEO218 presented a new property differ than 3-BP, it induced necrosis instead apoptosis if compared to the other hybrids. Still, it may overcome the limitations of 3-BP. Overall, the addition of POH with existing drugs, it is one of the most promising strategies to create potent molecules, capable of clinical treatment of patients, and that present a safe profile in all cases. Yet, the synthesis can be difficult and very dependent of the structure of the original drug.

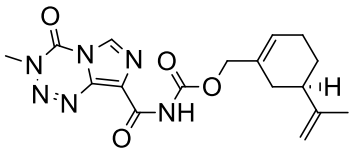
Table 4. Structure and range of activity of the hybrids with existing drugs



NEO214



NEO218



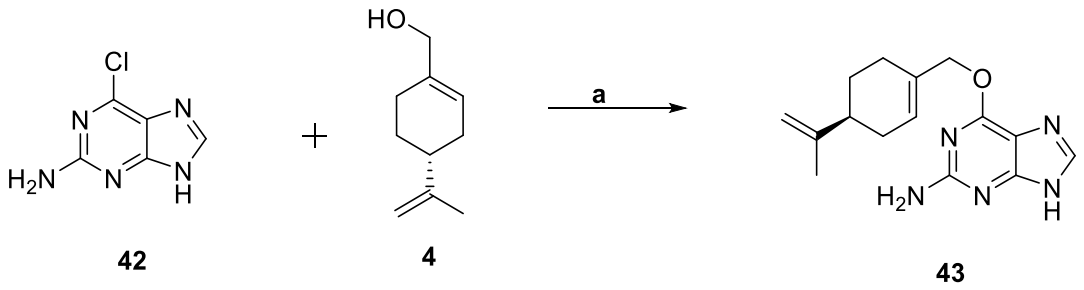
NEO212

Hybrid	Drug conjugated	RANGE OF IG_{50} OR IC_{50} (μM) Drug	RANGE OF IG_{50} OR IC_{50} (μM) HYBRID
NEO218	3-bromopiruvate	15-60a ; 150-300b	15-25ab
NEO214	Rolipram	551 – 1 mMc	50-59c
NEO212	Temozolomide	5-40d	10-18*d
Perillyl alcohol	**-**	**>1000 a-d**	**>1000 a-d**

*A = cell lines with high MCT-1, b = cell lines with low MCT-1, c = MM cell lines, d= U251, LN226, and TMZ resistant cell lines; *not effective in resistant cell lines*

3. Synthetic hybrids with specific chemical classes

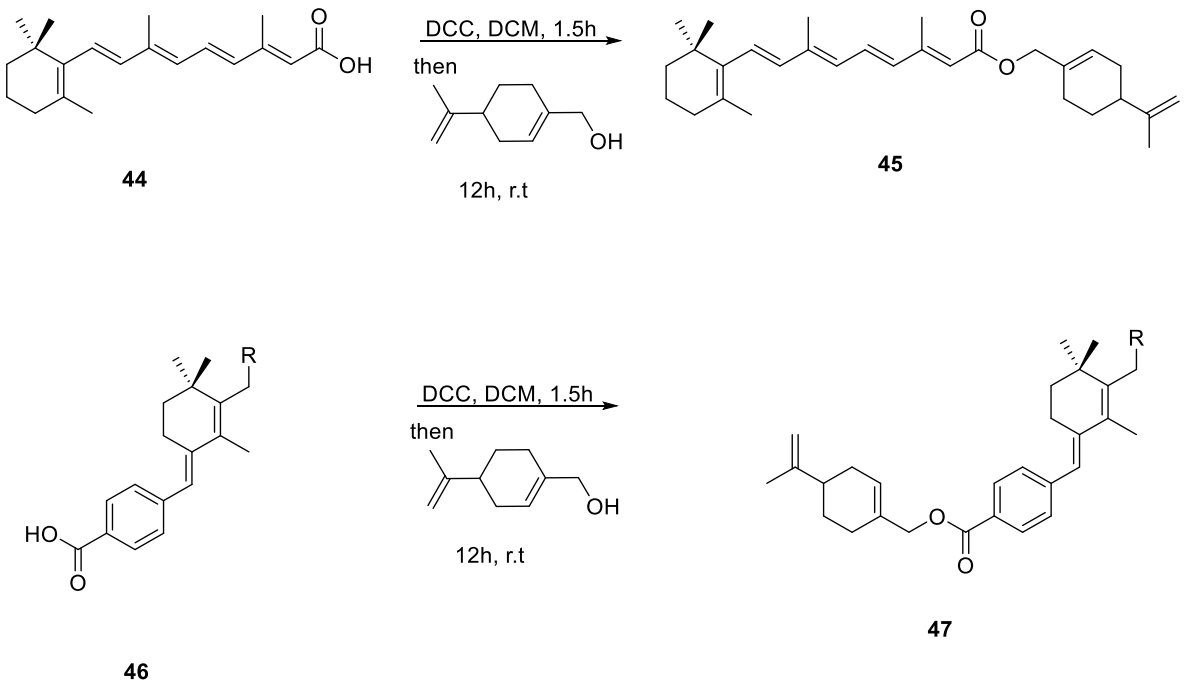
As evidence showed, POH hybrids are still a field that have a long way to explore. Beyond the use of existing drugs, some chemical classes attracted the interest in synthesis and development of new hybrids. One of the early reports to introduce perillyl alcohol was made by Griffin *et al.* in 2000 [61], where O6-Alkenyl-, O6-Cycloalkenyl-, and O6-(2-Oxoalkyl) guanines were synthesized with different substituents to target MGMT and increase sensitivity for Temozolomide (Scheme **8**). Although the hybrid **43** was very active for antiproliferative (around 2 μM) it was not the most of the series but represents an early example that POH can be used in conjunct of antiproliferative classes. The insertion of POH was made by classic Williamson ether reaction with alcohol and NaH in the presence of chloride, in this case 2-aminotrimethylpurin-6-ylammonium chloride to afford the final products. The scaffold based in the guanines and purines is still explored for antitumor activities [62].



42 **4** **43**

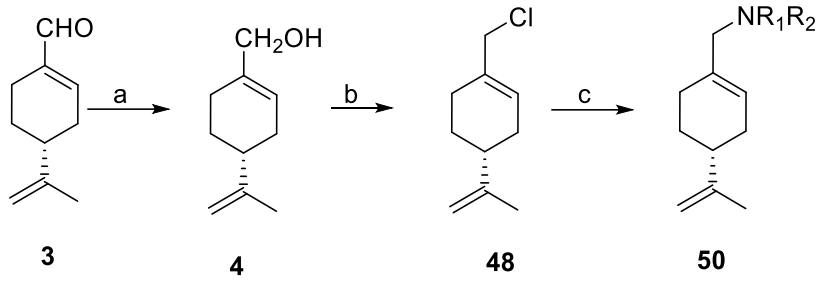
Scheme 8. Hybrids in the work of Griffin (2000). Reagents and Conditions: (a) NaH, THF, reflux.

Years later, Das *et al.* [63] proposed the synthesis of perillyl alcohol and novel retinoids (Scheme **9**), based in a strategy applied by Camps P. with hybrids for Alzheimer's disease, in 2010 [64]. As retinoids present an interesting class for treat cancer, and at the time, studies demonstrated the promising anticancer activities for perillyl alcohol, a hybrid was proposed. Besides anticancer, Retinoic acid hybrids are a topic of investigation in cell signaling and mechanisms [65].



Scheme 9. Synthesis of Retinoids in the work of Das (2010).

Later, this idea was also explored directly using amines by Hui *et al.* in 2014 [24], exploring both the isopropenyl portion of (S)-perillyl alcohol (discussed before) and the direct modification of the hydroxyl group in the upper part of the molecule (Scheme **10**). Amines are directly used for increase antiproliferative effect. In this case, POH is reduced with PPH_3 and CCl_4, making a conversion of hydroxyl group to a chloride **48**. This is a recurring synthetic pathway with mild conditions and acceptable yields (around 70%) and allows the amines to be inserted and other nucleophilic groups. As the main idea was to increase activity and to add new functionalities, the insertion of amines was a success in most of the compounds and presented a higher cytotoxic effect in the substitution of the hydroxyl portion than the isopropenyl. The most active compound being one with amantadine against A549, A375-S2 and HT1080 cell lines with IC_{50} in the range of 50-60 μM. Amantadine is administered as antiviral and antiparkinsonian [66] and this was the first time that was present in a structure of POH. Hui also verified if the activity was due to a synergic or addictive effect and the activity was solely based on this new synthesized structure.



Scheme 10. Synthesis of amino derivatives in hydroxyl portion by Hui (2014). Reagents and Conditions : (a) $NaBH_4$, EtOH, 0 °C-r.t., 3 h; (b) Ph_3P, CCl_4, CH_2Cl_2, r.t.; (c) R_1R_2NH, K_2CO_3, CH_3CN, reflux, 6–8 h.

This study indicated that the modification of hydroxyl portion is one of the most promising way to explore new derivatives. Inspired by these positive outcomes, Vendrusculo [67] presented a more robust and functionalized synthesis, as showed in Scheme **11**. As the previous hybrids presented good nonproliferation effects and numerous examples started to appear, a more complex synthesis take place. In this study was proposed, the unification of perillyl alcohol with dihydropyrimidinone by a triazole linker. The dihydropirimidinone class is present in some heterocycles that present a well-documented anticancer activity, such as monastrol [68, 69]. The use of a triazole linker was explored in other works for antiproliferative activity, such as the hybrids made by β-lactam and chalcone by Singh [70], and more recently by the substitution of the olefin bond by triazoles by Mustafa [71]. The synthesis of dihydropirimidinone are made via multicomponent Biginelli reaction, with urea, ethylacetoacetate and in this case propargyloxi benzaldehydes.

Synthetically the same approach by Hui was adopted: the conversion of perillyl alcohol to perillyl chloride with PPh_3 and CCl_4 (Appel reaction) and transformed in intermediary with azide **55**. The hybrids were linked together with perillyl azide **55** and propargyloxi dihydropyrimidinone **54**, by Huisgen reaction catalyzed by copper. For all the synthesized molecules, four presented an increased antiproliferative activity for some cell lines, and the presence of POH may increase the activity. The final yields of the products are variable with global yields around 50% to 81%.

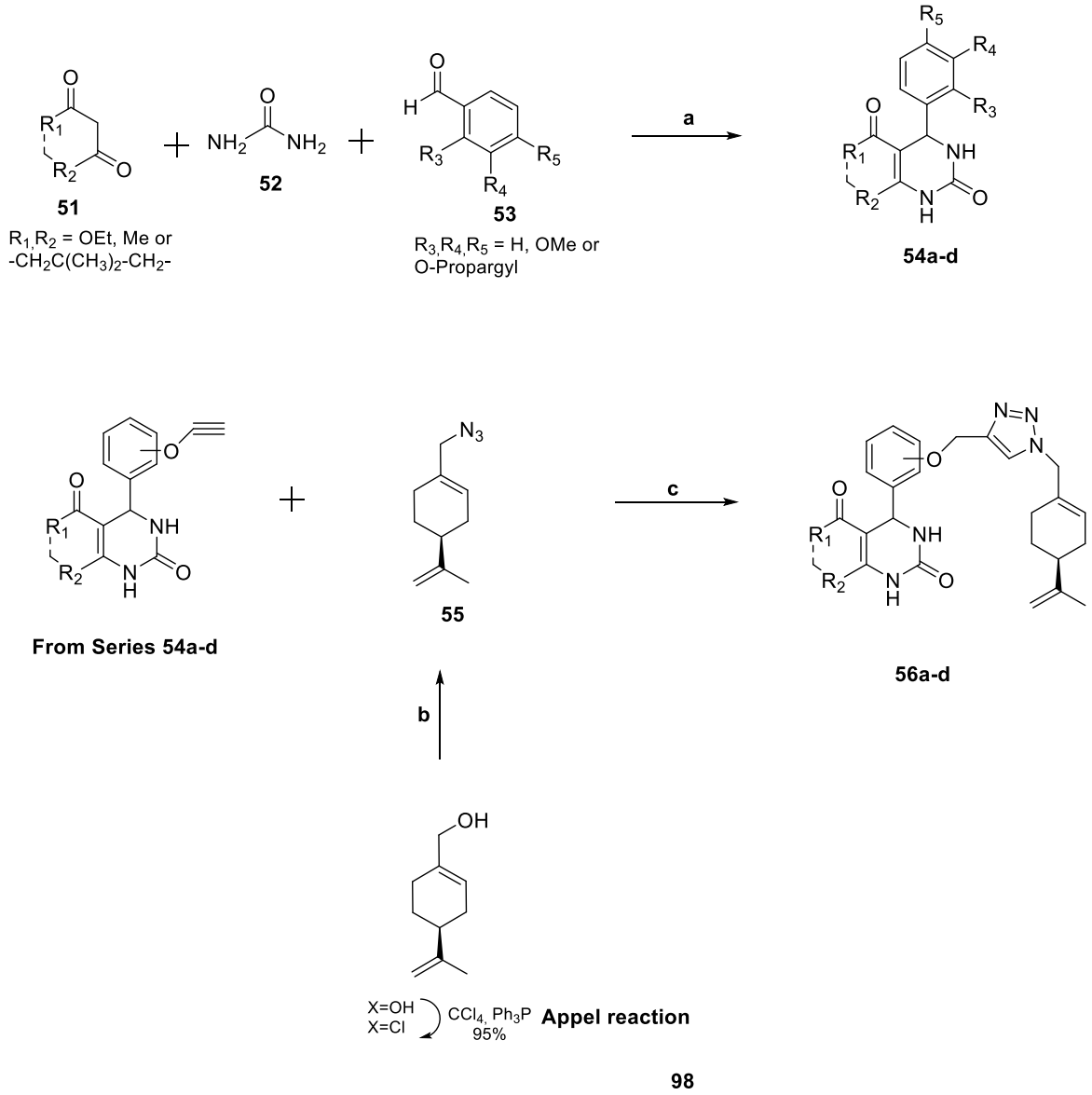
In the same year of 2018, other hybrid was proposed and synthesized by Said [72], with the introduction of perillyl alcohol and synthetic phenols linked together as

presented in Scheme **12**. This kind of approach (where a cyclic monoterpene) was also explored in other natural compounds such as myrtene and camphenol [73], and in comparison with other hybrids, the synthetic strategy takes another way: directly alkylation with other phenols, by Friedel-Crafts alkylation, with $BF_3.Et_2O$ as the catalyst.

This strategy is divergent even if the outcome is the same: the hybrids. The studies of Hui and Vendruscolo adopt the Appel reaction as a way to convert POH to perillyl chloride. This is largely dependent what it will be reacted (such as amines or azides). In the case of this study, the insertion of polyphenols were a direct strategy, not necessary to modify POH first, although the yields were regular. In all the synthesized polyphenols, the yields are variable between 21.4 % and 42.6%, with the obtention of the final product. In comparison with the Appel reaction, that presents an overall yield in both studies of Vendruscolo and Hui around 60%, and the final reaction to obtain the product, the use of direct alkylation may be a more direct strategy. Yet, in comparison with the previous works with global yields, the yields are lower. The use of $BF_3.Et_2O$ was an improvement over the use of aluminum phenoxide and aluminum isopropoxide in the alkylation of myrtenol.

As these studies largely vary in structure, the most active compounds presented a preference cell lines and are organized in Table **5**. The molecules presented by Hui have a sensitivity towards A549 cells for both most active molecules (with adamantine and cyclohexylamine) but the IC_{50} was not capable to produce *hit* molecules. More active molecules are presented by Vendrusculo with sensitive toward U251, UACC-62 cells with lower TGI values, for example for compound **56b** 3,8 μM. The chemical class explored by the author, dihidropyrimidinones, was extensile studied for their antiproliferative effects and one factor that increased this effect is the presence of perillyl alcohol. The same is attested in the latest work of Said, where synthetic phenols are attached with perillyl alcohol, with an increase in the antiproliferative activity. In this case, the sensitivity is for PC-3 cells similar to the modifications of limonene and for A529 cell lines, with activity below 50 μM, rendering these molecules as *hits*.

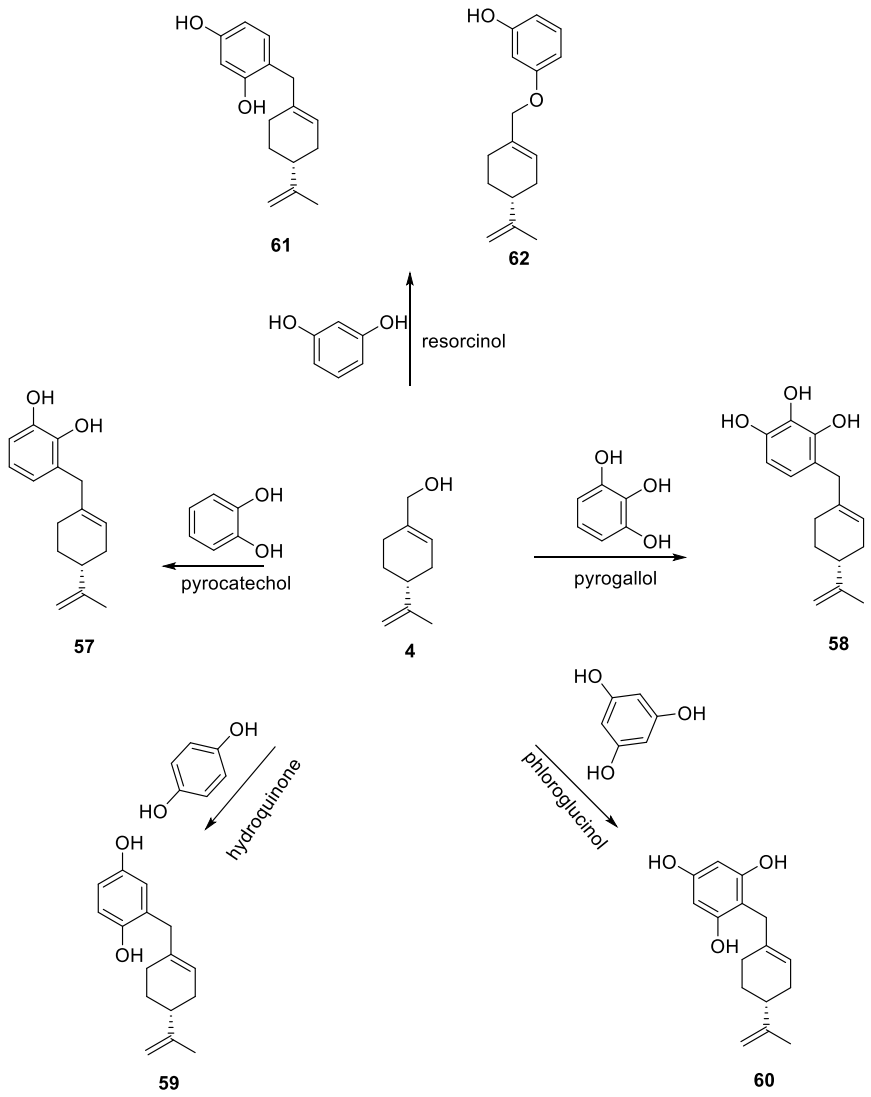
In all of these cases, the main conclusion is the same: perillyl alcohol when attached to these chemical classes enhanced the activity and triggered new mechanistic pathways regarding the cancer. This was observed especially in the last work of



Scheme 11. Limonene linked molecules in the work of Vendrusculo in 2018. Reagents and Conditions: (a) Lewis or Brownsted Acids, EtOH, reflux; (b) NaN_3, DMF, r.t, 12h; (c) $CuSO_4$, Na.Ascorbate, DCM/H_2O.

Said and in the hybrids proposed by Chen. Nevertheless, a special attention is required in regard of sensitivity for specific cell lines. In regards of synthesis, POH is usually treated as substituent that is attached and not as scaffold as in the examples of the isopropenyl group of limonene and POH. The yields in most of studies are regular to satisfactory with a short number of reactions to obtain the final product. .

The reactions are classic reactions (such as the Appel reaction), but other reactions can be explored. Vendrusculo presented specific and varied reactions, with the triazole linker by Huisgen and Bigineli reaction with acceptable yields between 40-50%.

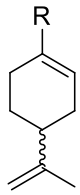


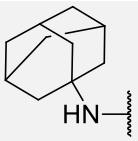
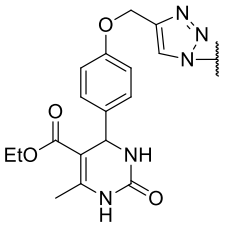
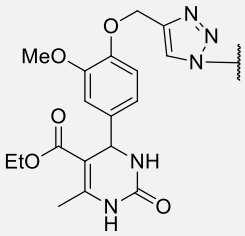
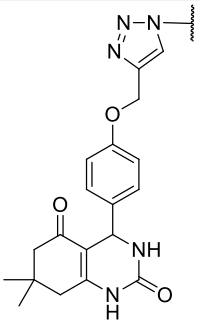
Scheme 12. Perillyl alcohol and polihydroxy derivatives. Reagents and Conditions: (a) $BF3{\cdot}OEt_2$, CH_3CN, N_2 at r.t. for 48 h

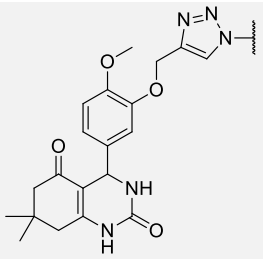
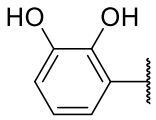
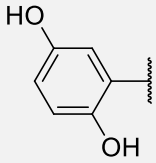
Further exploration of POH with other linkers, other promising chemical classes and even a multiple modification in both isopropenyl and hydroxyl portion could lead to promising new candidates for the treatment of cancer and increase the scope of study

of this very important terpene. In addition, the recent studies indicating that POH can be effective for Parkinson [74] and Alzheimer [75] is another field worth of exploration, which is in the beginning.

Table 5. Most active compounds in all works with synthetic and promising chemical classes.



COMPOUND	R	Configura-tion of pa	YIELDS (%)	RANGE OF IG_{50} OR TGI (μM)
50a		*(S)*	36.2	53.8a; 56.1b
56a		*(S)*	79	6.9c; 12.4d
56b		*(S)*	67	3.8e;48c
56c		*(S)*	68	6.6d; 23.9f

56d		*(S)*	79	8.2d; 44.8g
58		*(S)*	22	12.2g; 25.9f
59		*(S)*	28	20.5g; 37.0f

a = in A549 cell lines, b = in A375 cell lines, c = OVCAR3 cell lines, d = U251 cell lines, e = UACC-62 cells, f = PC3 cell clines, g = MCF7 cell lines

4. Conclusions

Limonene and its metabolite perillyl alcohol have an extensive background for anticancer and the use of Organic Synthesis is a valuable tool to improve the antitumor effect of these monoterpenes. Since the beginning of the new century, many works demonstrated the capacities of these derivatives using medicinal chemistry strategies with promising results. Limonene and perillyl alcohol could be important synthetic scaffolds to treat a myriad of diseases and improve life conditions in benefit of humankind.

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CONSIDERAÇÕES FINAIS

Com as informações dispostas nesta dissertação, novas contribuições podem ser dadas para a academia e a literatura nos temas apresentados. A síntese de novos candidatos a repelentes foi concluída e os testes preliminares demonstram boas perspectivas.

Foi considerado e aplicado em todo o trabalho, estratégias modernas e pouco exploradas até o momento neste tópico. Essas estratégias incluem a síntese assimétrica, e também uma posterior avaliação *in silico* das moléculas testadas. Com esses novos estudos, é possível esclarecer e afirmar que o uso de síntese melhora a repelência para compostos naturais, com resultados promissores.

A revisão sistemática dos derivados do limoneno e do álcool perílico organiza de forma coesa e sucinta este tema tão importante, que é o desenvolvimento de novos derivados que tem atividade anticâncer, que vem sido explorado largamente nos últimos anos utilizando estes derivados. Desta forma, toda a informação se mantém disposta em um *review.*

Futuramente, tem-se como perspectivas a conclusão dos testes de repelência com todos os derivados de forma a compreender a influência dos grupos funcionais na atividade, bem como partir para testes testando o possível efeito sinérgico destas moléculas, que possam melhorar ainda mais a repelência. Também serão realizados testes em relação a toxicidade e em pele humana. Será avaliado também o potencial destes derivados como larvicidas e inseticidas.