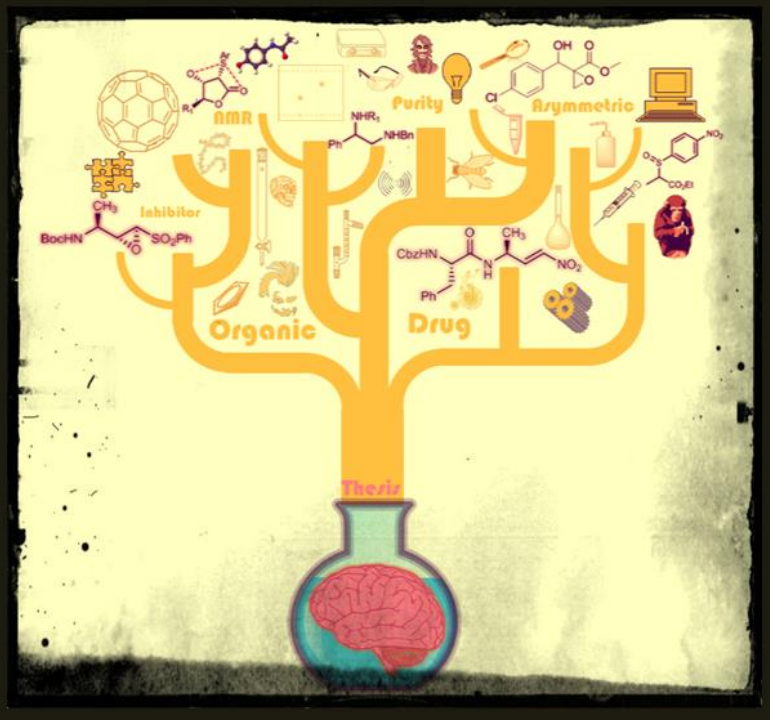
UNIVERSITAT JAUME·I

PhD THESIS 2015

Antonio Latorre Martínez

# STUDIES OF SULFURATED COMPOUNDS AND EPOXIDATION OF MBH ADDUCTS. NEW INHIBITORS OF CYSTEINE PROTEASES




**PhD THESIS**
**ANTONIO LATORRE MARTÍNEZ**

December 2015

**DIRECTORS:**
Florenci González Adelantado
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UNIVERSITAT JAUME·I

**Universitat Jaume I**

Escola Superior de Tecnologia i Ciències Experimentals
Departament Química Inorgànica i Orgànica
Grup Química Orgànica i Mèdica

# STUDIES OF SULFURATED COMPOUNDS AND EPOXIDATION OF MBH ADDUCTS. NEW INHIBITORS OF CYSTEINE PROTEASES

Tesis Doctoral
Antonio Latorre Martínez
Castellón 2015

Los directores Dr. Florenci Vicent González Adelantado y Dr. Santiago Rodríguez Pastor, profesores titulares del Departamento de Química Inorgánica y Orgánica de la Universitat Jaume I de Castellón,

**CERTIFICAN**: Que la tesis doctoral "STUDIES OF SULFURATED COMPOUNDS AND EPOXIDATION OF MBH ADDUCTS. NEW INHIBITORS OF CYSTEINE PROTEASES" ha sido desarrollada por Antonio Latorre Martínez, bajo su dirección, en el Área de Química Orgánica del Departamento de Química Inorgánica y Orgánica de la Universitat Jaume I.

Y para que así conste, en el cumplimiento de la legislación vigente y para los efectos oportunos, firman el presente certificado.

En Castellón, a 19 de Octubre de 2015.

Fdo.: Florenci V. González Adelantado Fdo.: Santiago Rodríguez Pastor

Esta tesis ha sido llevada a cabo gracias a una beca predoctoral concedida por la Universitat Jaume I (PREDOC/2009/45) dentro del plan de promoción de la investigación, desde el 1 de diciembre de 2010 hasta el 30 de noviembre de 2014. Además de una beca de colaboración de tercer ciclo de la Universitat Jaume I durante el año 2007 y 2008; y a un contrato de técnico superior de investigación para el grupo de química orgánica y médica con la fundación Genoma para el desarrollo y la investigación durante 2008 y 2009.

Antonio Latorre Martínez ha realizado una estancia en el *Leiden Institute of Chemistry*, de la *Leiden University* en los Países Bajos desde el 3 de septiembre hasta el 21 de diciembre de 2012, gracias a una ayuda concedida por la Universitat Jaume I dentro del plan de promoción de la investigación de 2012. El trabajo que se llevó a cabo llevaba por nombre "Diseño, síntesis y evaluación mediante ABPP de nuevos inhibidores de cisteína proteasas", bajo la supervisión de Dr. Hermen Overkleeft.

Esta tesis ha sido realizada y será defendida de acuerdo con los requisitos exigidos para la obtención del título de Doctorado Internacional.

Previamente a la defensa de la Tesis Doctoral, este trabajo ha sido evaluado por dos expertos internacionales independientes, directamente relacionados con el área de investigación de la presente Tesis Doctoral y de reconocido prestigio internacional: Prof. Dr. Tanja Schirmeister del departamento de Farmacia y Bioquímica de la *Mainz University,* Alemania y Dr. Jean-Luc Decout del departamento de Química Molecular y Farmacoquímica de la *University Joseph Fourier/Grenoble*, Francia.

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Vicentín

*“D’ací endavant meu”*

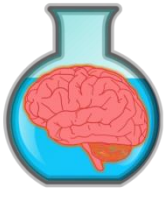
# Abbreviations

ABP = activity based probe
ABPP = affinity based protein profiling
Bodipy = boron-dipyrromethene
Chx = cyclohexyl
CID = collision induced dissociation
CMK = chloromethyl ketone
*m*-CPBA = *meta*-chloroperoxybenzoic acid
DABCO = 1,4-diazabicyclo[2.2.2]octane
DCM = dichloromethane
DM = Dess Martin Periodinane
DMSO = dimethylsulfoxide
d.r. = diastereoselectivity ratio
EDC = 1-etil-3-(3-dimetilaminopropil)carbodiimida
Ei = internal elimination
EP = epoxide
ESI = electrospray ionization
eV = electronvolt
EWG = electron withdrawing group
FMK = fluoromethyl ketone
h = hour
HIV = human immunodeficiency virus
HMK = halomethyl ketone
HMPA = hexamethylphosphoramide
HOBt = benzotriazol-1-ol
MBH = Morita-Baylis-Hillman
min = minute
MS = mass spectrometry
mp = melting point
*m/z* = mass-to-charge ratio
NMM = *N*-methylmorpholine
NMR = nuclear magnetic resonance
NOE = nuclear Overhauser effect
o.n. = over night
Q or q = quadrupole
PAGE = polyacrylamide gel electrophoresis
PEG = polyethylene glycol
py = pyridine

PFTBA = perfluorotri-*n*-butylamine
r.t. = room temperature
S = Svedberg sedimentation coefficient
SDS = sodium dodecyl sulfate
TBPLi = lithium *tert*-butylperoxide
TBS = *tert*-butyldimethylsilyl
TEA = triethylamine
TFA = trifluoroacetic acid
Tempo = 2,2,6,6-tetramethyl-1-piperidinyloxy
THF = tetrahydrofuran
TOF = time of flight
Tol = toluene
VS = vinyl sulfone
WHO = World Health Organization

# Index

# General introduction

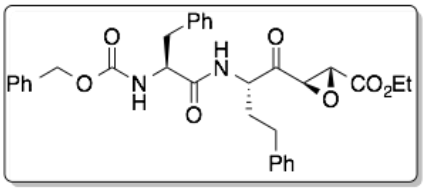


The Organic and Medicinal Chemistry Group at University Jaume I has developed several works during the last few years. These works defined a research line about organic chemistry goals and a different PhD studies for this years.

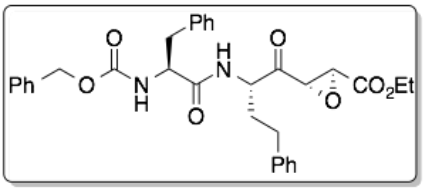
As a result of this group research, some publications were available and determined the investigation road for last years. These group publications can be divided in cysteine proteases and asymmetric synthesis as follows.

Cysteine proteases inhibitors publications:

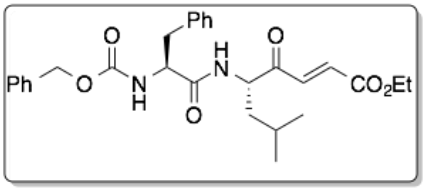
1. Dipeptidyl-α,β-epoxyesters as potent irreversible inhibitors of the cysteine proteases cruzain and rhodesain.



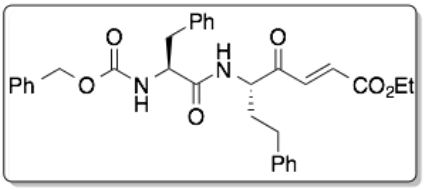
Cruzain $IC_{50}$ = 20nM ; $k_{inact}/K_I$ = 82900 $M^{-1}s^{-1}$
Rhodesain $IC_{50}$ = 3.5nM ; $k_{inact}/K_I$ = 92090 $M^{-1}s^{-1}$



Cruzain $IC_{50}$ = 50nM ; $k_{inact}/K_I$ = 25200 $M^{-1}s^{-1}$
Rhodesain $IC_{50}$ = 30nM ; $k_{inact}/K_I$ = 23500 $M^{-1}s^{-1}$



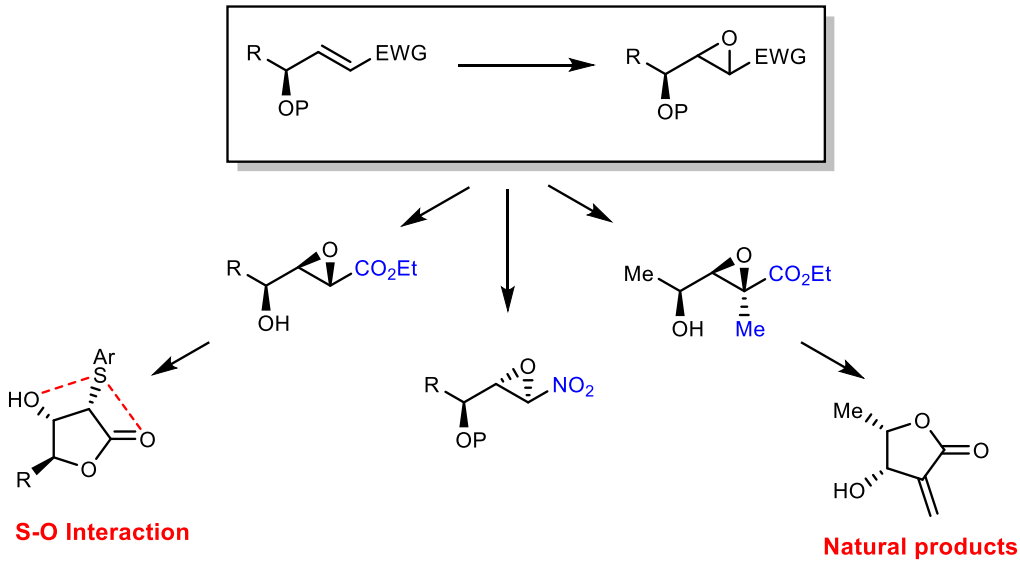
Rhodesain $IC_{50}$ = 16.4nM ; $k_{inact}/K_I$ = 1610000 $M^{-1}s^{-1}$



Rhodesain $IC_{50}$ = 26nM ; $k_{inact}/K_I$ = 1530000 $M^{-1}s^{-1}$

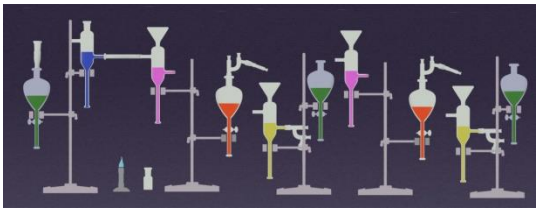
Asymmetric synthesis and derivatization:

1. Stereoisomerization of β-Hydroxy-α-sulfenyl-γ-butyrolactones controlled by two concomitant 1,4-Type nonbonded sulfur-oxygen interactions as analyzed by X-ray crystallography.
2. Highly stereoselective epoxidation of α-methyl-γ-hydroxy-α,β-unsaturated esters: rationalization and synthetic applications.
3. Diastereoselective synthesis of γ-hydroxy-α,β-epoxyesters and their conversion into β-hydroxy α-sulfenyl γ-butyrolactones.
4. Asymmetric epoxidation of poor-electron olefins.




According to these group experience and the interest fields of the group, this thesis was focused on all of these fields with new studies as summarized in the next chapter of objectives of the thesis.

# Objectives



English version

This thesis was focused in all of the projects described above. During these studies, the main objective was to increase the experience and the skills in the organic chemistry laboratory. These fields are enough different to use very different kind of reactions, reagents, characterizations and techniques allowing to know more chemistry than working in a single field. The main objectives were divided in the following fields:

Cysteine proteases inhibitors

1. Design new cysteine proteases inhibitors against parasitic diseases and proteasome.
2. Develop a quick and easy route to obtain the designed new inhibitors and control the modifications to make a wide scope for each family.
3. Evaluate these new inhibitors against desired enzymes.
4. Once evaluated the inhibition activity, redesign the structure inhibitors to increase the activity and make a new family of inhibitors.

Asymmetric synthesis and derivatization

1. Explore compounds and reactions with new S-O interactions.
2. Study new chiral poor-electron olefins compounds and the stereoselectivity in the epoxidation
3. Study of mechanistic pathways of interesting transformations

The progress of this investigation on these fields is explained in the following chapters and gave rise to new publications for our research group.

Versión española

Teniendo en cuenta los campos de investigación descritos en la introducción general en los que tiene interés nuestro grupo de investigación, la tesis ha estado centrada en desarrollar todas y cada una de las líneas de investigación. Durante las investigaciones llevadas a cabo en esta tesis, el principal objetivo ha sido desarrollar la experiencia y la suficiencia investigadora, ampliando las aptitudes y conocimientos relacionados con la química orgánica.

Los campos de investigación en los que se ha desarrollado la tesis son suficientemente diferentes, por la naturaleza de las reacciones, sustratos, reactivos y técnicas de caracterización que ha servido para ampliar más el conocimiento que habiéndose dedicado el tiempo a un solo campo.

Los principales objetivos quedan resumidos en los siguientes puntos:

Inhibidores de cisteína proteasa:

1. Diseñar nuevos inhibidores de cisteína proteasa contra enfermedades parásitas y proteasoma.
2. Desarrollar rutas sintéticas rápidas y versátiles para obtener estos nuevos inhibidores, teniendo en cuenta la posibilidad de ampliar el número de compuestos por familia.
3. Evaluar en primera persona estos nuevos inhibidores contra los enzimas deseados.
4. Con los resultados de inhibición, plantear y rediseñar las estructuras de estos compuestos para obtener una nueva generación mejorada de inhibidores.

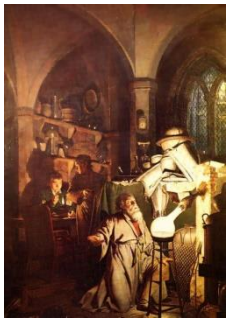
Síntesis asimétrica y derivatizaciones

1. Buscar nuevos compuestos con nuevas interacciones S-O.
2. Llevar a cabo el estudio de la estereoselectividad de olefinas quirales deficientes en electrones.
3. Hacer estudios exhaustivos de los mecanismos de reacción de transformaciones químicas interesantes.

El progreso de estas investigaciones en cada uno de los campos mencionados va a ser explicados en los capítulos siguientes y ha dado lugar publicaciones.

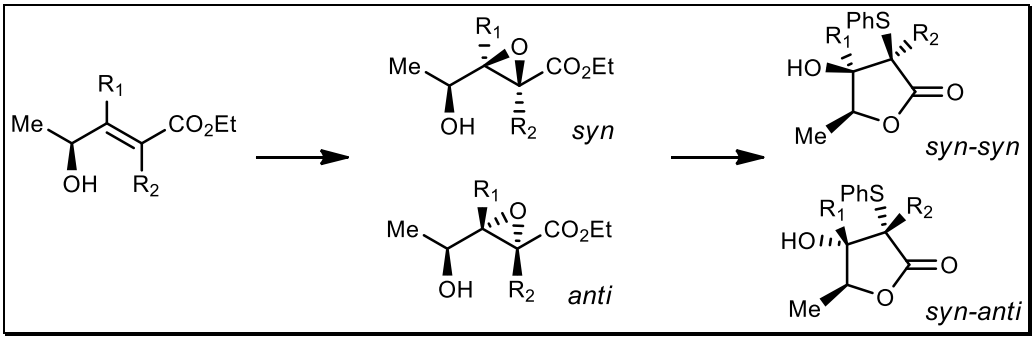
# Chapter 1:

# Study of the mechanism in the elimination of 2-arylsulfinyl esters



# 1. Introduction

During our previous study of the epoxidation of α-methyl-γ-hydroxy-α,β-unsaturated esters, two butyrolactones were obtained (*syn-syn* and *syn-anti*)[1,2] as represented in **scheme 1**.

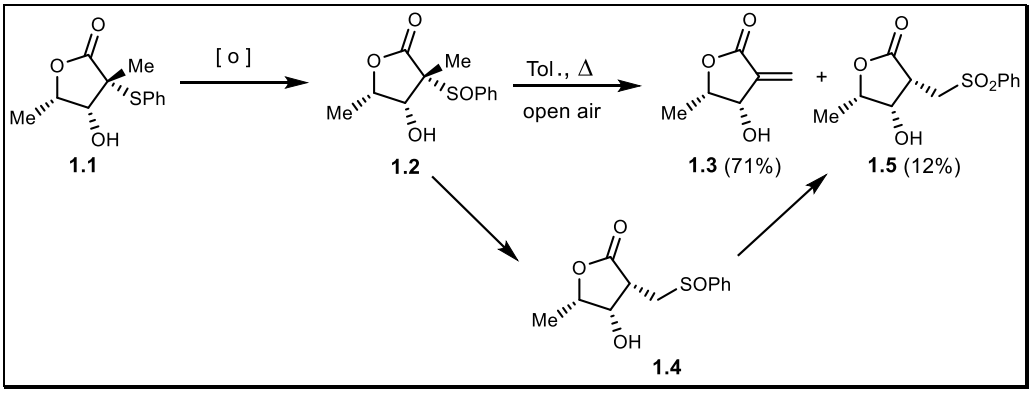



**Scheme 1.** Previously obtained butyrolactones.

[1] López, I.; Izquierdo, J.; Rodríguez, S.; González, F. V. *J. Org. Chem.* **2007**, 72, 6614–6617
[2] González, F. V.; Jain, A.; Rodríguez, S.; Sáez, J.; Vicent, C.; Peris, G. *J. Org. Chem.* **2010**, 75, 5888– 5894

As a further application of these compounds, synthesis of natural product **1.3** was carried out. After sulfur oxidation of selected butyrolactone *syn-anti* to the corresponding sulfoxide, elimination reaction was carried out under refluxing toluene in open air. The spectroscopy data for compound **1.3** were identical to those described in the literature.[3,4]

During this transformation, the attention was focused in the mechanism of elimination of 2-arylsulfinyl esters to form the alkene. Curiously, sulfone **1.5** was also obtained.




**Scheme 2:** Formation of rearrangement product **1.5**

The **scheme 2** shows the elimination reaction of sulfoxide **1.2** to give exomethylenic compound **1.3** as the main reaction product and sulfone **1.5** as a minor product. Sulfone **1.5** results from an oxidative rearrangement of sulfoxide **1.2** in a highly stereoselective fashion across the sulfoxide **1.4**. The structure of sulfone **1.5** was determined by single crystal X-ray diffraction and it is shown in **figure 1**. Presumably, β-oxo sulfoxide **1.2** rearranges to a γ-oxo sulfoxide **1.4** which upon oxidation furnishes γ-oxo sulfone **1.5**. It has been reported the high tendency

[3] Martínez, J. C. V.; Yoshida, M.; Gottlieb, O. R. *Phytochemistry* **1981**, 20, 459-464
[4] Hanson, R. L.; Lardy, H. A.; Kupchan, S. M. *Science* **1970**, 168, 376-380

of thioethers with neighboring hydroxyl groups to be oxidized to sulfones instead of sulfoxides.[5]

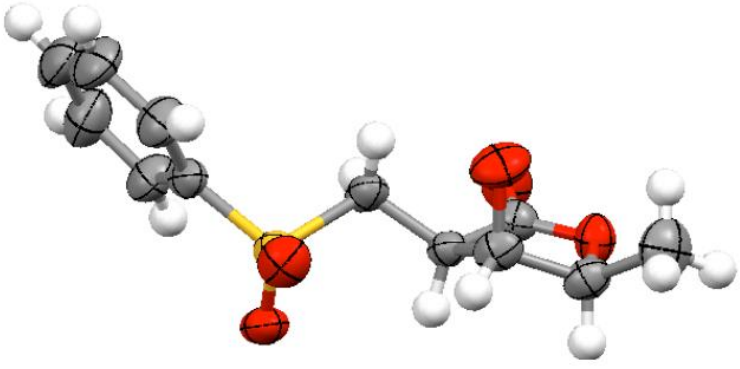
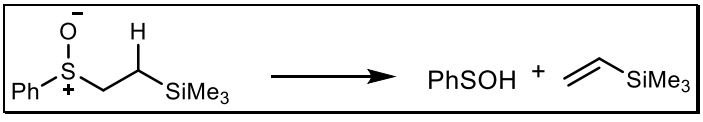


**Figure 1:** ORTEP representations (50 % probability ellipsoids) of compound **1.5**

Since Cram and Kingsbury studied the mechanism of sulfoxides elimination and proposed an internal elimination mechanism (Ei),[6] the elimination reactions of sulfoxides and sulfones are commonly applied in organic chemistry,[7] especially in the case of 2-arylsulfinyl esters with the aim of obtaining unsaturated carbonyl compounds.[8,9,10,11] An example of eliminations to obtain alkenes can be observed in **scheme 3**.

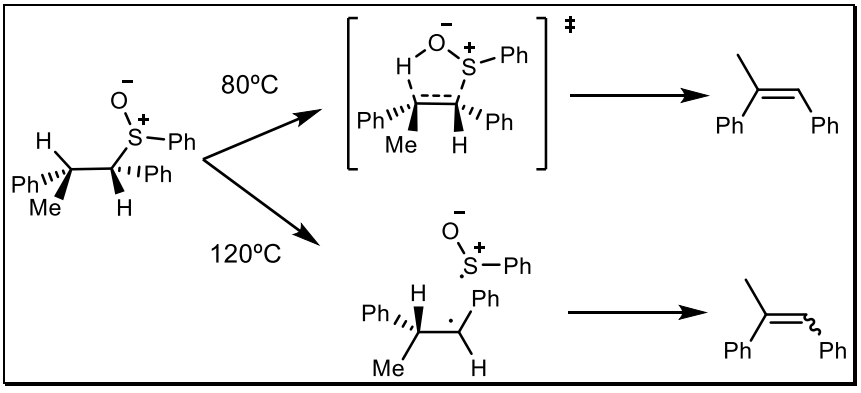



**Scheme 3:** Example of elimination of sulfoxides

[5] Clennan, E. L.; Kang, Y. *J. Org. Chem.* **1992**, 57, 4477– 4487
[6] Kingsbury, C. A.; Cram, D. J. *J. Am. Chem. Soc.* **1960**, 82, 1810–1819
[7] Carreño, C. *Chem. Rev.* **1995**, 95, 1717–1760
[8] Trost, B. M.; Salzmann, T. N.; Hiroi, K. *J. Am. Chem. Soc.* **1975**, 97, 4887–4902
[9] Trost, B. M.; Leung, K. K. *Tetrahedron Lett.* **1975**, 48, 4197–4200
[10] Trost, B. M.; Mao, M. K. T.; Balkovec, J. M.; Buhlmayer, P. *J. Am. Chem. Soc.* **1986**, 108, 4965–4973
[11] Bänziger, M.; Kleina, S.; Rihs, G. *Helv. Chim. Acta* **2002**, 85, 1399–1406

In the above mentioned work,[6] authors studied the elimination of 1,2-diphenyl-1-propyl phenyl sulfoxides and suggested also a competing homolytic mechanism. This mechanism would operate when the radicalary intermediate can be stabilized as for 1,2-diphenyl-1-propyl phenyl sulfoxides. They reached to this supposition based on the loss of stereospecificity in some products when the reaction was performed at high temperature (**scheme 4**).

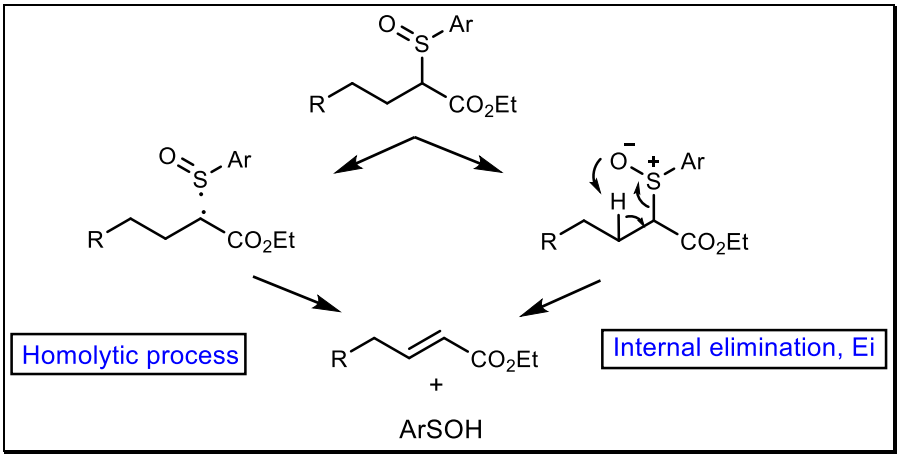
Although sulfoxide elimination reaction has been widely applied,[12,13] no further evidence of this homolytic mechanism involving a sulfinyl group has been reported in the literature.




**Scheme 4:** Theoretical pathways proposed in literature[12]

Based upon the mechanistic pathways suggested in literature,[12] two possible routes can be considered for the transformation of 2-arylsulfinyl esters into the unsaturated compound: the concerted elimination reaction and the radical process (**scheme 5**).

[12] Cubagge, J. W.; Guo, Y.; McCulla, R. D.; Jenks, W. S. *J. Org. Chem.* **2001**, 66, 8722-8736
[13] McCulla, R. D.; Jenks, W. S. *J. Org. Chem.* **2003**, 68, 7871– 7879



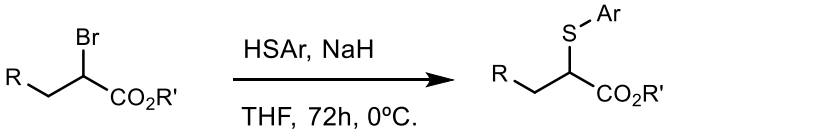

**Scheme 5:** Mechanistic pathways for the elimination of 2-arylsulfinyl esters

The main difference between two pathways is the existence of a radical intermediate, in the second one. Then, our efforts were focused in the confirmation of this intermediate in the reaction media by capturing the intermediate with a radical scavenger in the chemical reaction and by mass spectrometry techniques.

# 2. Results and discussion

For getting a wider scope in this study, a family of 2-arylsulfinyl esters with a range of alkyl and aryl groups (R) was prepared.

2-Arylsulfinyl esters were prepared from 2-bromo esters in two steps. The treatment of commercially available 2-bromo esters with the corresponding thiolate, previously prepared by combining the corresponding thiol and sodium hydride in tetrahydrofuran as a solvent cold with an ice bath for 72 h, afforded the thioethers **1.6a-f** in good yields, as it can be observed in **table 1**.



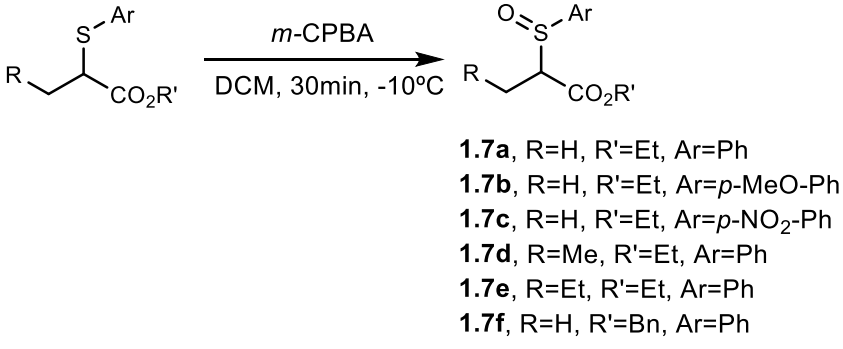

**1.6a**, R=H, R'=Et, Ar=Ph
**1.6b**, R=H, R'=Et, Ar=*p*-MeO-Ph
**1.6c**, R=H, R'=Et, Ar=*p*-$NO_2$-Ph
**1.6d**, R=Me, R'=Et, Ar=Ph
**1.6e**, R=Et, R'=Et, Ar=Ph
**1.6f**, R=H, R'=Bn, Ar=Ph

**Scheme 6:** Preparation of thioethers **1.6a-f**

Table 1. Preparation of thioethers

| Entry | Product | R | R' | Ar | Yield (%) |
|---|---|---|---|---|---|
| 1 | **1.6a** | H | Et | Ph | 65 |
| 2 | **1.6b** | H | Et | *p*-MeO-Ph | 60 |
| 3 | **1.6c** | H | Et | *p*-$NO_2$-Ph | 52 |
| 4 | **1.6d** | Me | Et | Ph | 93 |
| 5 | **1.6e** | Et | Et | Ph | 88 |
| 6 | **1.6f** | H | Bn | Ph | 70 |

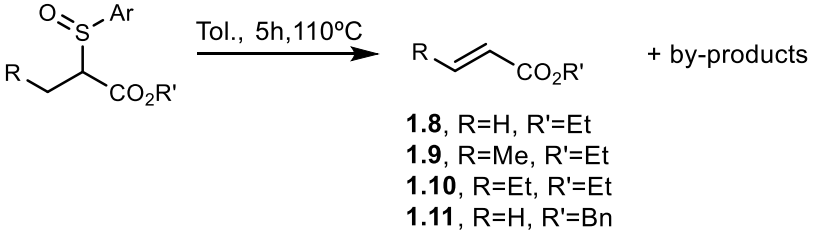
The thioethers were oxidized with *m*-CPBA in dichloromethane at -10 °C for 30 minutes, affording the corresponding sulfoxides **1.7a-f** in good yields as shown in **table 2**.




**Scheme 7:** Preparation of sulfoxides **1.7a-f**

| Table 2. Preparation of sulfoxides | | | | | |
|---|---|---|---|---|---|
| **Entry** | **Product** | **R** | **R'** | **Ar** | **Yield (%)** |
| 1 | **1.7a** | H | Et | Ph | 83 |
| 2 | **1.7b** | H | Et | *p*-MeO-Ph | 51 |
| 3 | **1.7c** | H | Et | *p*-$NO_2$-Ph | 62 |
| 4 | **1.7d** | Me | Et | Ph | 82 |
| 5 | **1.7e** | Et | Et | Ph | 92 |
| 6 | **1.7f** | H | Bn | Ph | 70 |

The elimination reaction was done as shown in **scheme 8**, by heating at reflux in toluene for five hours. In all cases only *trans* isomer was isolated and any trace of *cis* isomer was observed by NMR, even when the reaction was carried out at 80 °C.

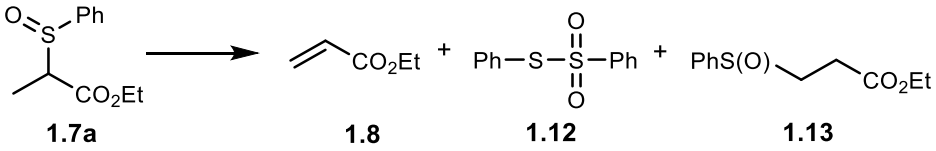



**Scheme 8:** Elimination reaction

Elimination compounds were characterized as the main reaction products and their data had fully consonance with the physical data from literature.[14,15,16,17]

[14] *Aldrich Library of 13C and 1H FT NMR spectra*; Aldrich: Milwaukee; 1993, p 973C
[15] Nishizawa, M.; Hirakawa, H.; Nakagawa, Y.; Yamamoto, H.; Namba, K.; Imagawa., H. *Org. Lett.* **2007**, 9, 5577–5580

The crude reaction was complex as detected by NMR in all cases. Minor reaction products were phenylthiosulfonate **1.12** and sulfoxide **1.13** (**scheme 9**). Then a careful purification of the reaction mixture was carried out.




**1.7a** **1.8** **1.12** **1.13**

**Scheme 9:** Elimination reaction products

The sulfoxide **1.13** is a reaction product equivalent to the rearranged lactone **1.4.** This interesting transformation could be an alternative synthetic route to prepare 3-arylsulfinyl esters.

## 2.1. Mechanism study

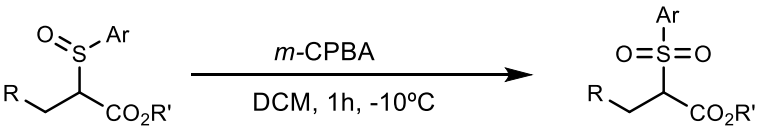
In order to know in detail the mechanism in the elimination of 2-arylsulfinyl esters, a whole study was carried out. An important aspect of the study was to find out if the mechanism follows a homolytic pathway.

### 2.1.1. Elimination of sulfonyl and sulfenyl esters

The first point was to determine if sulfonyl esters and sulfenyl esters can also eliminate as sulfinyl esters do. Sulfonyl esters were prepared from previously

[16] Kandula, S. R. V.; Kumar, *P. Tetrahedron: Asymmetry* **2005**, 16, 3268– 3274
[17] Pittelkow, M.; Christensen, J. B. *Org. Lett.* **2005**, 7, 1295– 1298

prepared thioethers. Sulfones were oxidized with *m*-CPBA in dichloromethane for 1h, shown in **scheme 10**. The yields obtained are included in **table 3**.




**1.7a**, R=H, R'=Et, Ar=Ph
**1.7b**, R=H, R'=Et, Ar=*p*-MeO-Ph
**1.7c**, R=H, R'=Et, Ar=*p*-$NO_2$-Ph
**1.7d**, R=Me, R'=Et, Ar=Ph
**1.7e**, R=Et, R'=Et, Ar=Ph
**1.7f**, R=H, R'=Bn, Ar=Ph

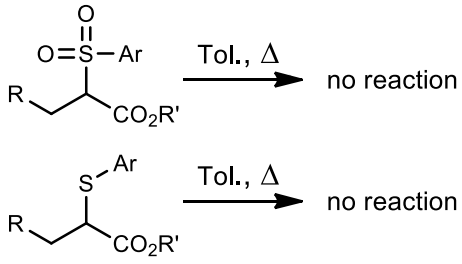
**1.14a**, R=H, R'=Et, Ar=Ph
**1.14b**, R=H, R'=Et, Ar=*p*-MeO-Ph
**1.14c**, R=H, R'=Et, Ar=*p*-$NO_2$-Ph
**1.14d**, R=Me, R'=Et, Ar=Ph
**1.14e**, R=Et, R'=Et, Ar=Ph
**1.14f**, R=H, R'=Bn, Ar=Ph

**Scheme 10**: Preparation of sulfonyl compounds

**Table 3. Preparation of sulfones**

| Entry | Product | R | R' | Ar | Yield (%) |
|---|---|---|---|---|---|
| 1 | **1.14a** | H | Et | Ph | 76 |
| 2 | **1.14b** | H | Et | *p*-MeO-Ph | 69 |
| 3 | **1.14c** | H | Et | *p*-$NO_2$-Ph | 61 |
| 4 | **1.14d** | Me | Et | Ph | 89 |
| 5 | **1.14e** | Et | Et | Ph | 92 |
| 6 | **1.14f** | H | Bn | Ph | 83 |

When those 2-arylsulfenyl esters and 2-arylsulfonyl esters were submitted to the elimination reaction under refluxing toluene for five hours, starting materials were recovered in all cases. The result (**scheme 11**), determined that only sulfinyl compounds can be converted to unsaturated carbonyl compounds by thermal treatment in toluene.

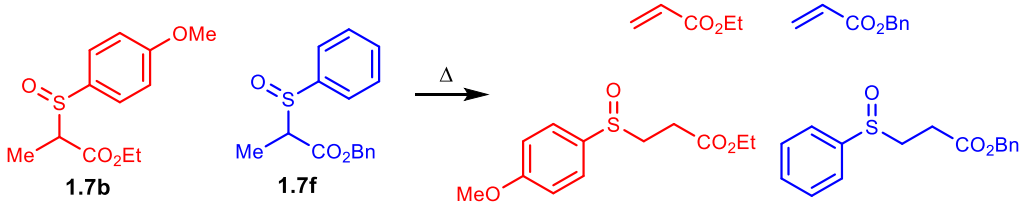



**Scheme 11**: Elimination reaction of sulfones and thioethers

### 2.1.2. Crossover

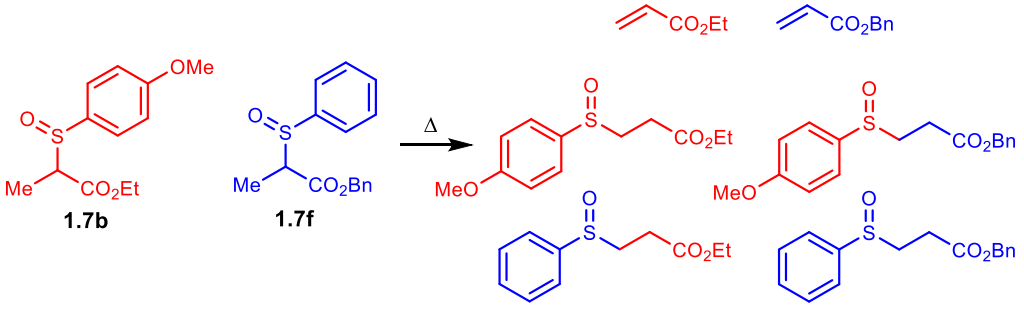
In order to determine if the formation of the rearranged 3-sulfinyl esters during the elimination of 2-arylsulfinyl esters results from an intramolecular process or it is intermolecular, a mixture of two different 2-arylsulfinyl esters (**1.7b** and **1.7f**) was submitted to elimination conditions and the reaction products were identified.

In the crossover experiment only two possibilities could be expected. The first one, illustrated in **scheme 12**, if the reaction was an intramolecular mechanism, only two sulfinyl compounds must be detected.



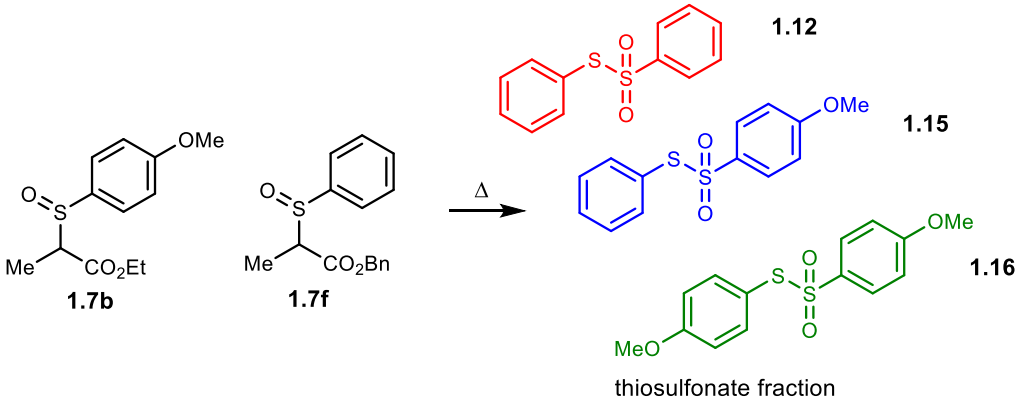

**Scheme 12:** Theoretical products via intramolecular pathway

On the other hand, if the reaction was an intermolecular mechanism four 3-arylsulfinyl compounds can be obtained, as it is shown in **scheme 13**.




**Scheme 13:** Theoretical products via intermolecular pathway

The mixture of compound **1.7b** and **1.7f** was refluxed in toluene for five hours and a complex crude reaction was obtained. All volatiles compounds were removed (ethyl acrylate **1.8** and benzyl acrylate **1.11**) and a separation by chromatography led to two fractions, thiosulfonate esters and sulfoxides products. The fraction with all thiosulfonate compounds was analyzed by $^{1}$H-NMR and $^{13}$C-NMR and very complex spectra were obtained. These fractions were also analyzed by electrospray ionization mass spectrometry, operating in positive mode (ESI +). The results obtained, unraveled a rapid and direct snapshot of three formed species for the thiosulfonate fraction (**scheme 14**), and four formed species for the sulfoxides (**scheme 15**).




**Scheme 14:** Elimination reaction, thiosulfonate fraction

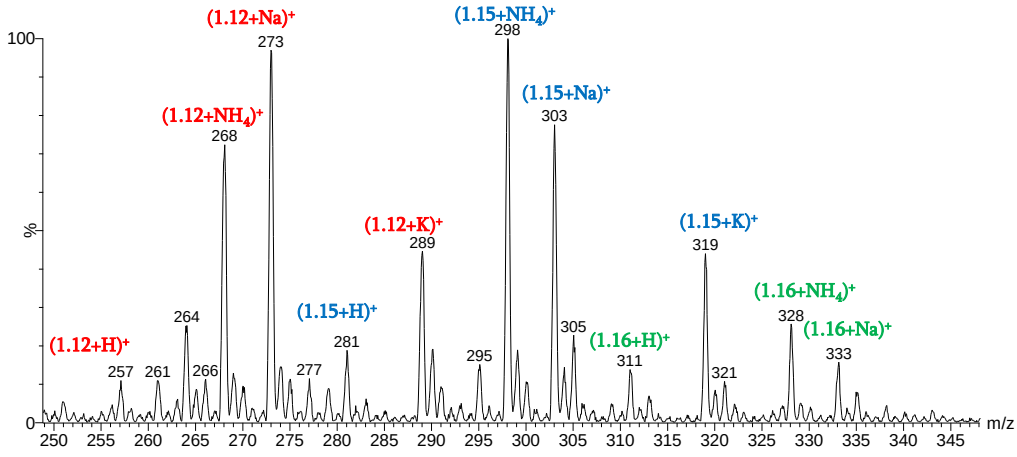
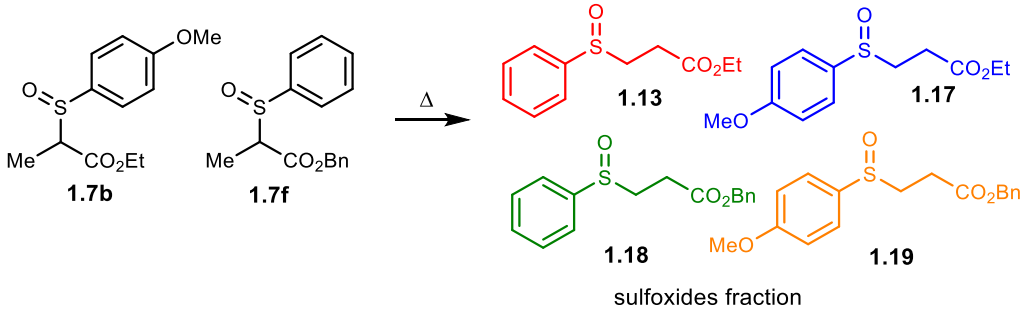



**Figure 2:** ESI-MS of the thiosulfonates fraction




**Scheme 15:** Elimination reaction, sulfoxides fraction

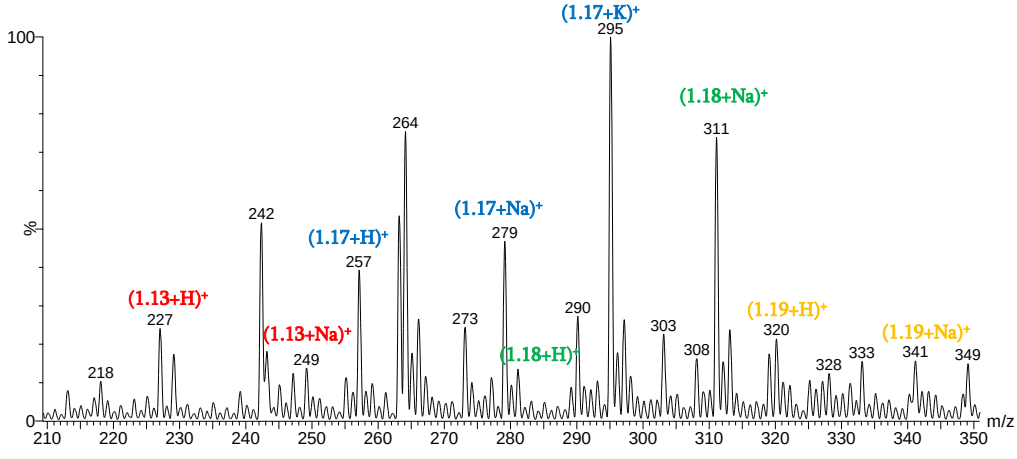


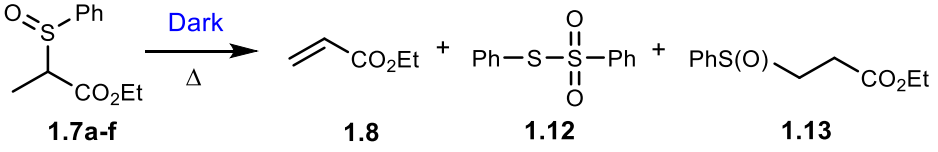

**Figure 3:** ESI-MS of the sulfoxides fraction

The presence of these crossover products can only be explained by a scission of the C-S bond, demonstrating the process to be intermolecular. When same reaction was carried out at 80 °C same results were obtained.

### 2.1.3. Reaction carried out in darkness

It has been reported that a carbon-sulfur bond can be cleaved via photolysis.[18] With the aim of confirming the role of light in the reaction, 2-arylsulfinyl esters **1.7a-f** were refluxed in toluene for five hours but in the absence of light, summarized in **scheme 16**.

The results of the reactions were in all cases the same than in the presence of light, demonstrating that light is not the cause of the carbon-sulfur cleavage.




**1.7a-f** **1.8** **1.12** **1.13**

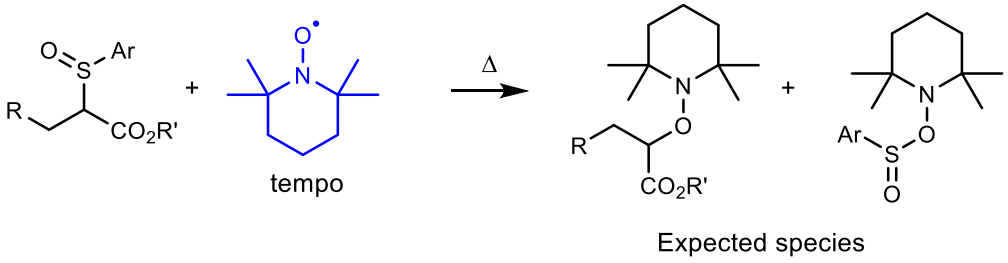
**Scheme 16**: Elimination in the absence of light

### 2.1.4. Trapping intermediates

Although the crossover experiments indicates the participation of free radicals, it was convenient to confirm by a direct way the presence of free radicals in the reaction process. The strategy was based on the use of the tapping agents as the 2,2,6,6-tetramethyl-1-piperidinyloxy (tempo), which is a stable nitroxyl

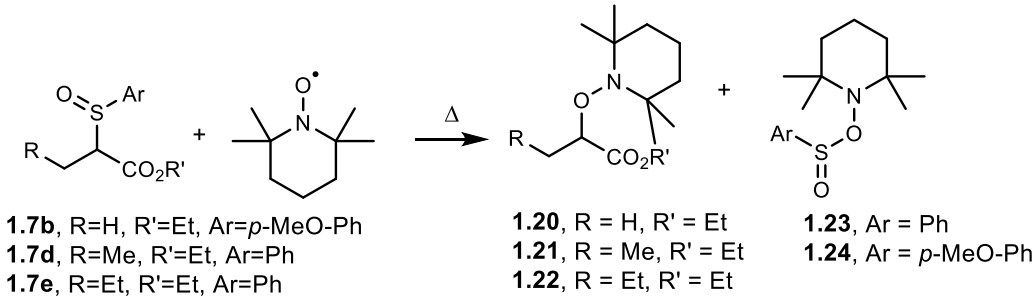
[18] Guo, Y.; Jenks, W. S. *J. Org. Chem.* **1997**, 62, 857– 864

radical, and it could react with the radical intermediates to form new species in the reaction crude.[19] The presence of these new species would provide a strong and clear evidence of the radical mechanism. The expected products are shown in **scheme 17**.




**Scheme 17**: Theoretical expected products

The typical elimination reaction was carried out with sulfoxides **1.7b**, **1.7d** and **1.7e** in toluene for five hours in the presence of tempo at 80 °C. The reaction crude was carefully concentrated and investigated by ESI-MS. Compound **1.7b** afforded adducts **1.20** and **1.24**, compound **1.7d** afforded adducts **1.21** and **1.23** and compound **1.7e** afforded **1.22** and **1.23**, as expected. The results can be seen in **scheme 18**.



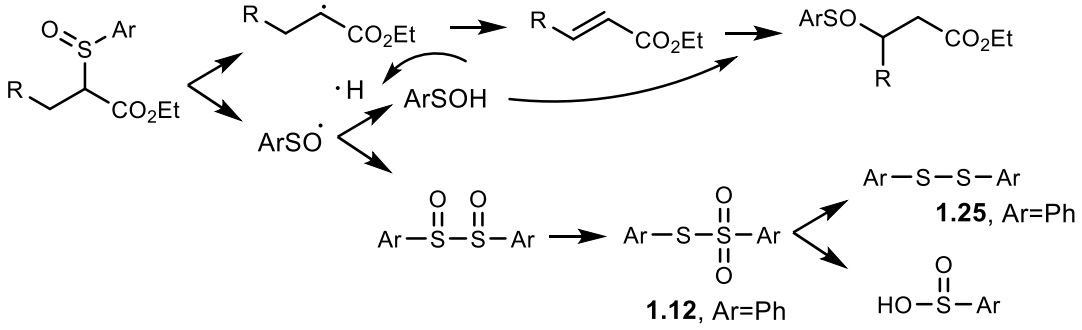

**Scheme 18**: Elimination reactions in the presence of tempo

[19] Zhang, X.; Wang, H.; Guo, Y. *Rapid Commun. Mass Spectrom.* **2006**, 20,1877– 1882

Only in the case of reaction of compound **1.7e** was possible to do a full characterization of products **1.22** and **1.23**. The complicated characterization of nitroxides as **1.20** are based on their reactivity as radical initiators,[20] for this reason the trapping reaction at 110 °C were not useful for compounds **1.20** and **1.21**. In that case, the reaction was performed at 80 °C; isolation and characterization were done to confirm the radical trapping product.

With all these experiments and products, the radical mechanism for the elimination of aryl sulfinyl esters into enoates was demonstrated. The presence of ester which can stabilize the radical intermediate, allowed the radical pathway. The detection and characterization of tempo trap produces an organic evidence of the intermediates of the radical mechanism.

The radical mechanism, complementing previous **scheme 9**, was proposed as depicted in **scheme 19**, according to the results and the reaction products.




**Scheme 19**: Radical mechanism pathway

Sulfoxides could give the sufinyl radical and the corresponding stabilized ester radical in a first step. Sulfinyl radicals combine to give *vic*-disulfoxides and

[20] Braslau, R.; Burrill II,L.C.; Siano, S.; Naik, N.; Howden, R.K.; Mahal, L.K.; *Macromolecules*, **1997**, *30* (21), 6445–6450

undergo to isomerization to form thiosulfonate. This product can partially be transformed into the diaryl sulfide and the sulfonic acid.

These results demonstrated a radical mechanism for the elimination for this range of sulfoxides into enoates. Information of the intermediates in a multistep reaction is important to confirm the mechanism pathway.[21]

## 2.2. Electrospray ionization tandem mass spectrometry

### 2.2.1. ESI mass spectrometry

The electrospray ionization (ESI), operating in positive (+) or negative (-) mode, coupled to mass spectrometry is usually used in organic chemistry (sometimes coupled to liquid chromatography), to determine the masses of different compounds in a desired sample or reaction crude.

A general scheme of the technique is illustrated in **figure 4**, the sample is ionized by an ESI source. This ionization is caused by a high voltage applied to a solvent containing the sample, to form an aerosol. After a nebulization and complete evaporation of solvent, sample analytes are charged and pushed by gas to the quadrupole.

In the quadrupole, ions can be modulated and separated according to the *m/z* value because of the magnetic field generated.

[21] Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry*; Kluwer Academic/Plenum Publishers: New York, **2000**; Part A, pp 226.

Finally these ions can be represented in a spectrum after the detector, giving important information about the masses of the molecules in the initial sample.

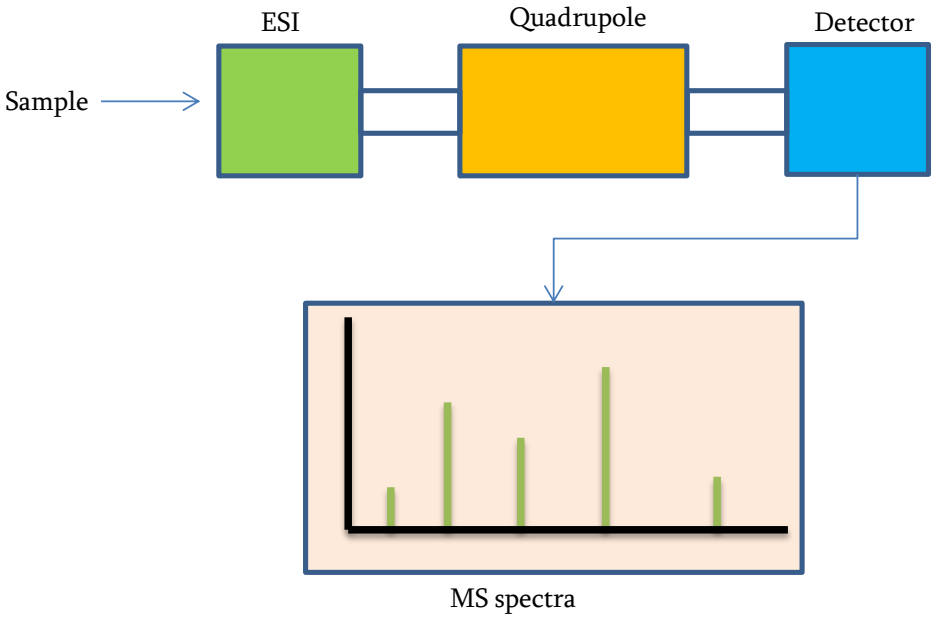



**Figure 4**: ESI mass spectrometry

Based on this technique, the study started by heating a solution of **1.7a** at 100 °C, different time aliquots were extracted, diluted with acetonitrile and analyzed by ESI(+) mass spectrometry. The spectrum of compound **1.7a** in an initial solution is shown in upper panel of **figure 5** and after 60 min in the lower panel of **figure 5**.

Both spectra obtained were nearly identical, only the appearance of new signals corresponding to the $PhSSO_2Ph$ **1.12** product was detected. This evidence was confirmed by the presence of the peaks assigned to $(PhSSO_2Ph+Na)^+$ (*m/z* 273) and $(PhSSO_2Ph+K)^+$ (*m/z* 289).

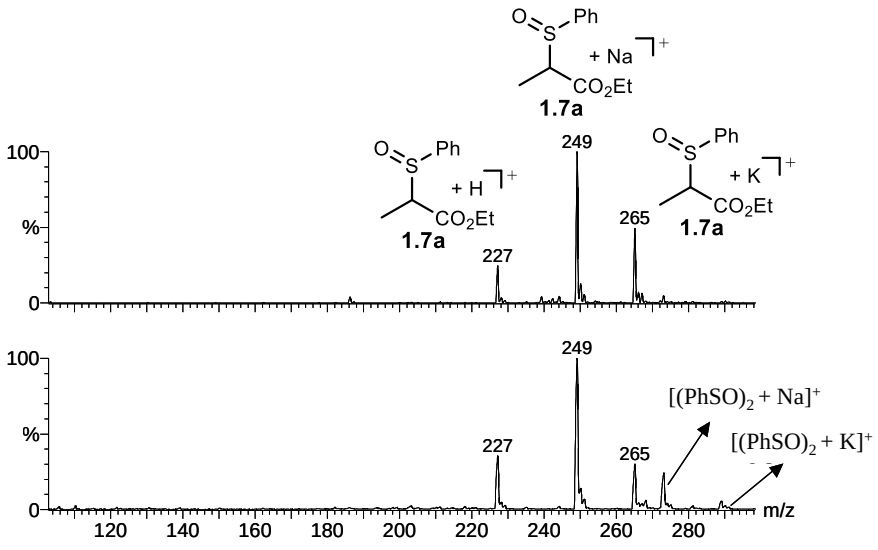



**Figure 5**: ESI(+) mass spectra

In ESI(-) mass spectrometry, only sulfonic acid $(M\text{-}H)^-$ was detected as expected. No evidence of diphenyldisulfide **1.25** was found as it was not easy to ionize in ESI (+ or -) conditions.

On the other hand, the presence of these radical intermediates could not be identified by ESI mass spectrometry directly. Because of that, it was decided to perform experiments in tandem by working with ESI MS/MS and monitoring the conversion of **1.7a** into ethyl acrylate **1.8**.

### 2.2.2. Tandem ESI/MS

The electrospray ionization tandem mass spectrometry (ESI-MS) is a technique which can be applied to determine intermediates in chemical reactions and biochemical processes.[22,23,24]

It can be considered extended version of the ESI mass spectrometry since two more quadrupoles are operating for the same experiment. In the **figure 6** is represented the scheme for this technique. It operates in the same way as before in the ESI mass spectrometry but a determinate *m/z* is selected (in Q1) to enter in the second quadrupole (q2). This q2 is usually working as a collision cell, collision induced dissociation (CID) takes place when a collision gas breaks the selected *m/z* ion in fragments. These new fragments ion are again modulated and separated according to the *m/z* values in the third quadrupole (Q3). Finally the detector can provide a spectrum of new compound present after the fragmentation.

It is remarkable CID, in determinate cases, contribute with essential information about the structure and reactivity of a desired compound.

[22] Santos, L. S.; Knaack, L.; Metzger, J. O. Int. *J. Mass Spectrom.* **2005**, 246, 84–104
[23] Eberlin, M. N. Eur. *J. Mass Spectrom.* **2007**, 13, 19– 28
[24] Santos, L. S. *Eur. J. Org. Chem.* **2008**, 235– 253

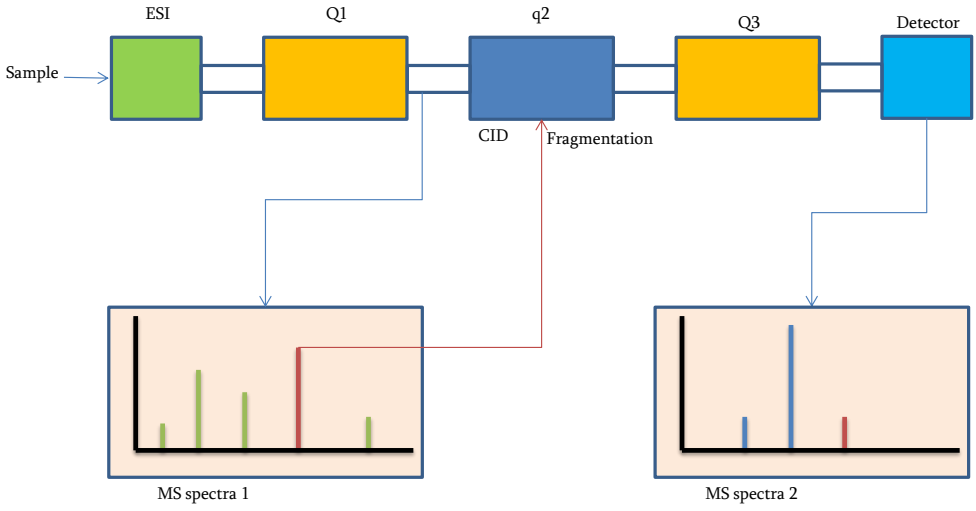



**Figure 6**: Tandem ESI mass spectrometry

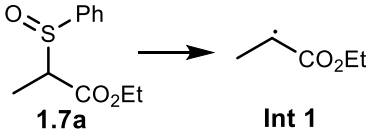
Free radicals are difficult to detect due to instability and the fast transformation into other compounds. This technique was recently used to determine radical intermediates in some processes related with thermally driven homolytic reaction.[25,26,27,28,29,30]

According to these results, attempts to intercept these radical intermediates were carried out based on the online tandem ESI-MS and MS/MS. MS/MS is an important technique because provide information about the structure of the compound for a selected *m/z.*

In that tandem experiment, the selected *m/z* was 249, corresponding to (**1.7a**+Na)$^+$ and (**1.13**+Na)$^+$, displayed distinctive unimolecular dissociation upon collision-induced dissociation (CID) conditions. The *m/z* 249 (**1.7a**+Na)$^+$ adduct was chosen to monitor the temporal reaction evolution.

[25] Meyer, S.; Koch, R.; Metzger, J. O. *Angew. Chem., Int.* Ed. **2003**, 42, 4700–4703
[26] Griep; Raming, J.; Metzger, J. O. *Anal. Chem.* **2000**, 72, 5665– 5668
[27] Meyer, S.; Metzger, J. O. *Anal. Bioanal. Chem.* **2003**, 377, 1108– 1114
[28] Fürmeier, S.; Metzger, J. O. *J. Am. Chem. Soc.* **2004**, 126, 14485– 14492
[29] Zhang, X.; Liao, Y.; Qian, R.; Wang, H.; Guo, Y. *Org. Lett.* **2005**, 7, 3877– 3880
[30] Schäfer, M.; Drayb, M.; Springer, A.; Zacharias, P.; Meerholz, K. *Eur. J. Org. Chem.* **2007**, 5162– 5174

In the case of **1.7a** *m/z* 249 (**1.7a**+Na)$^+$, predominantly dissociated via C-S bond cleavage affording the radical (**Int 1**+Na)$^+$ (*m/z* 124) represented in **scheme 20** and **figure 7.** On the other hand, compound **1.13** with *m/z* 249 (**1.13**+Na)$^+$ dissociated via the formation of (**1.8**+Na)$^+$ (*m/z* 123), as represented in **scheme 21** and **figure 8**.




**Scheme 20**: Dissociation of compounds **1.7a**

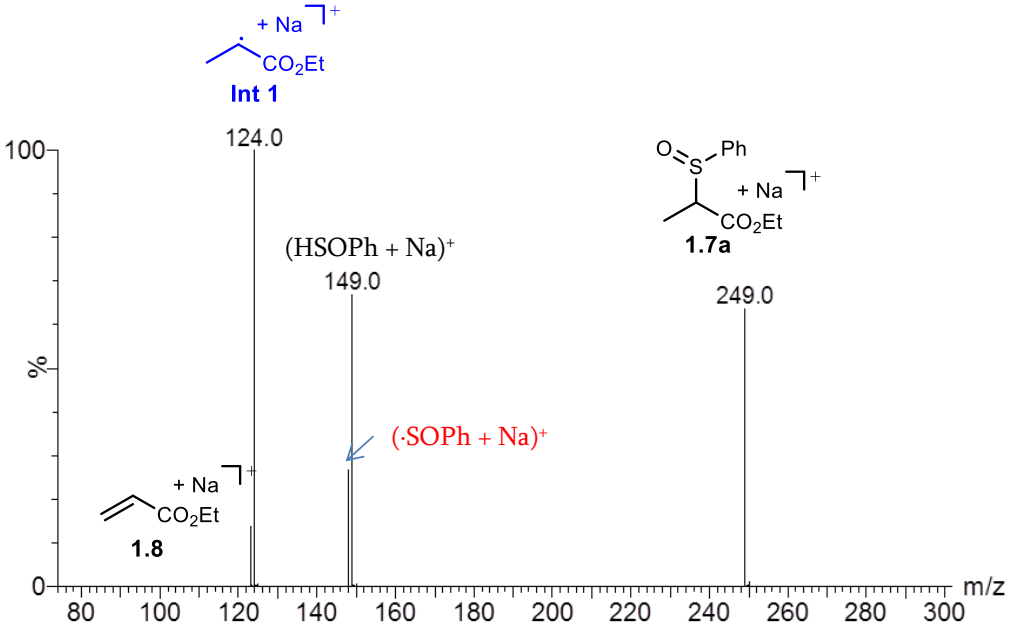
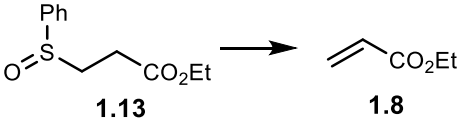



**Figure 7**: Dissociation spectrum of compound **1.7a**




**Scheme 21**: Dissociation of compound **1.13**

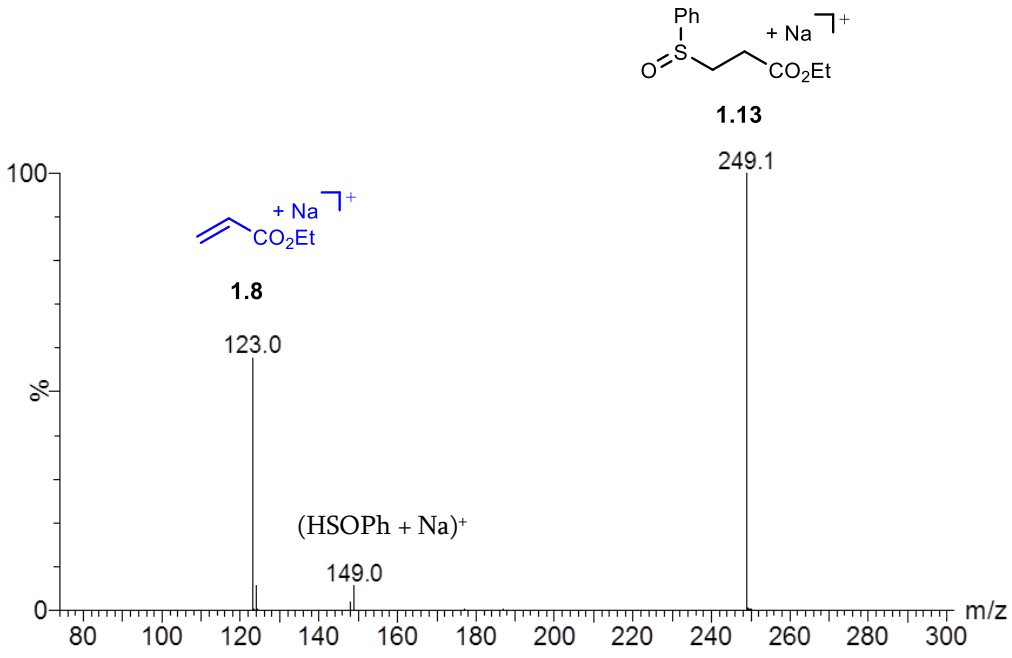
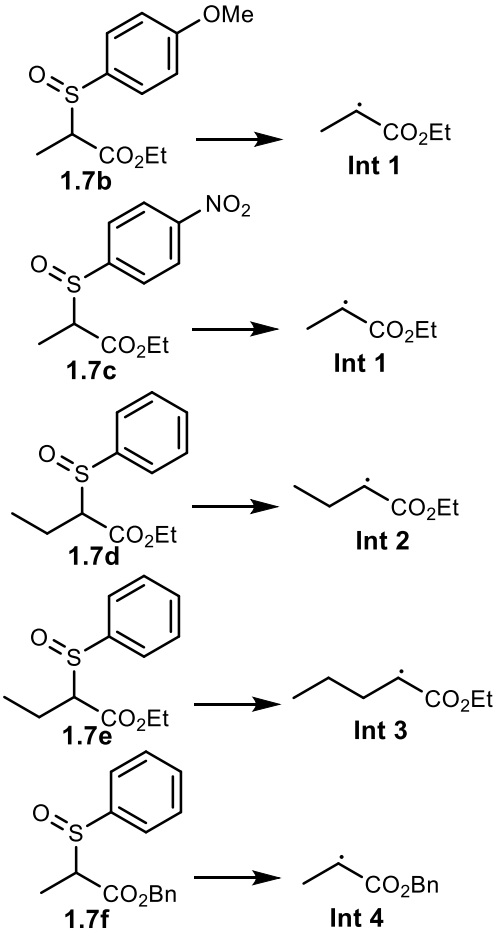



**Figure 8**: Dissociation spectrum of compound **1.13**

According to the collision energies, when the same energy was used (15eV) the intensity of the dissociated signal from (**1.7a**+Na)$^+$ was greater than signals from (**1.13**+Na)$^+$ demonstrating that species from **1.7a** dissociated easier than from **1.13**. The gas-phase behavior of **1.7a** was reminiscent of that observed for heated solutions; then this compound was more prone to homolytically dissociate via C-S bond cleavage.

Same results were obtained when compounds **1.7b-f** were monitored in the evolution of the reaction. In all cases the homolytic cleavage of C-S bond was the detected by the radical intermediate **Int 1-4** as in the previous case, illustrated in **scheme 22** and **figures 9-13**.




**Scheme 22**: General compound obtained for compound **1.7b-f**

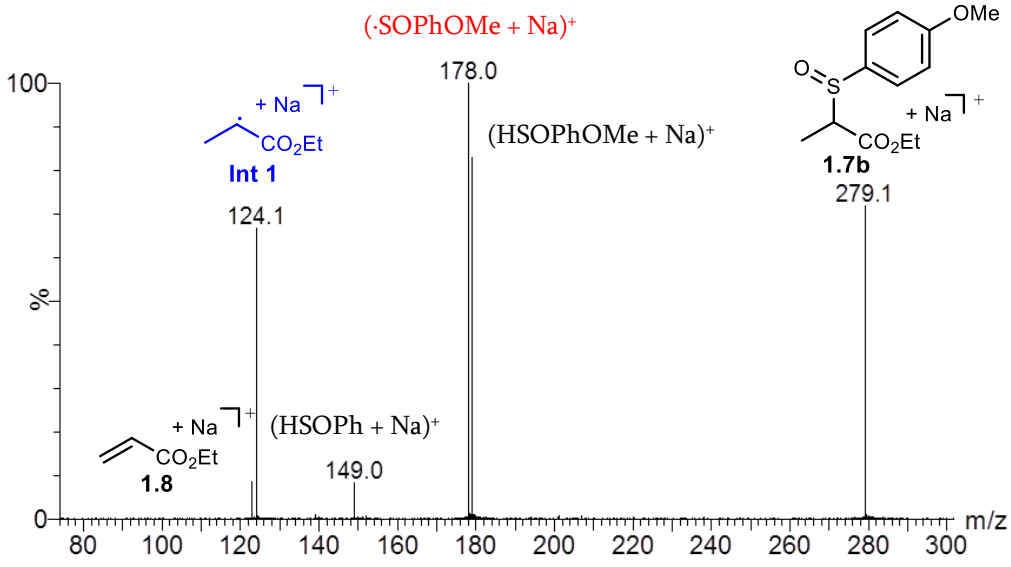



**Figure 9:** Dissociation spectrum of compound **1.7b**

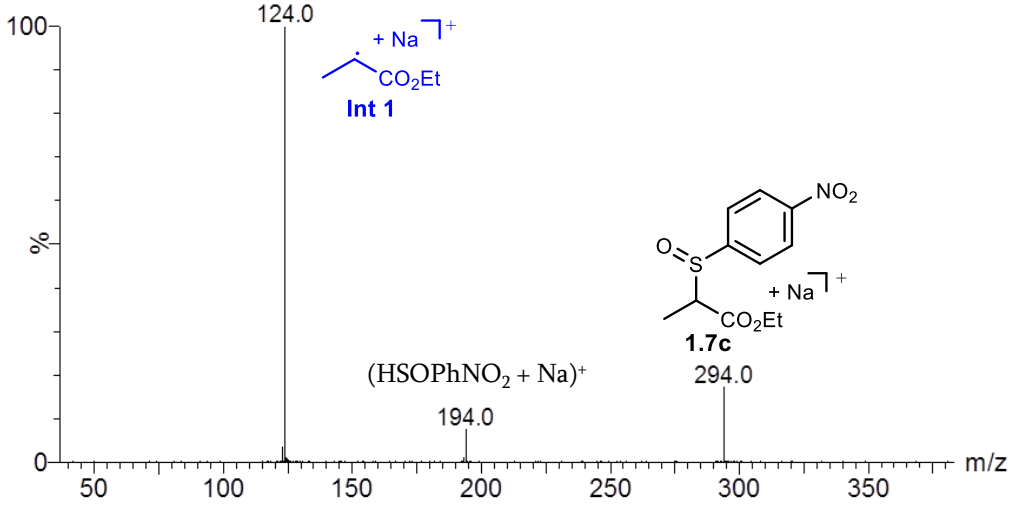



**Figure 10:** Dissociation spectrum of compound **1.7c**

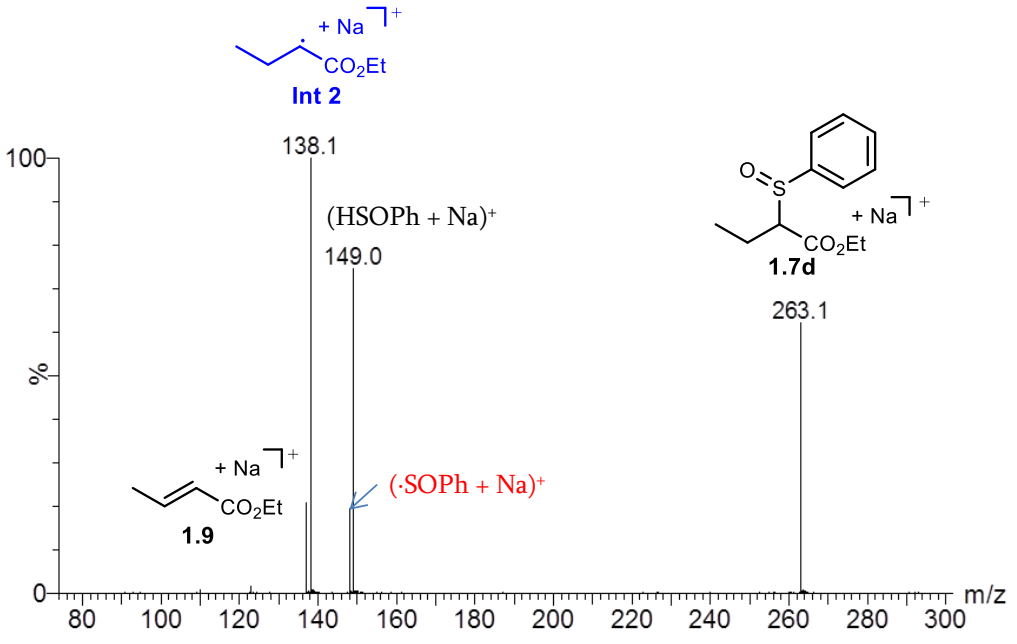



**Figure 11:** Dissociation spectrum of compound **1.7d**

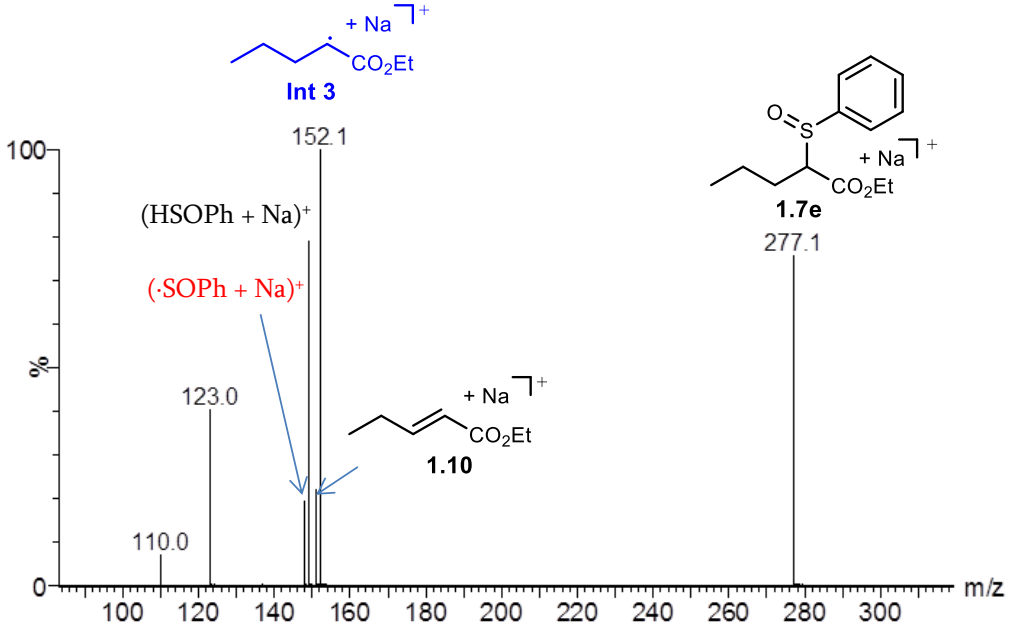



**Figure 12:** Dissociation spectrum of compound **1.7e**

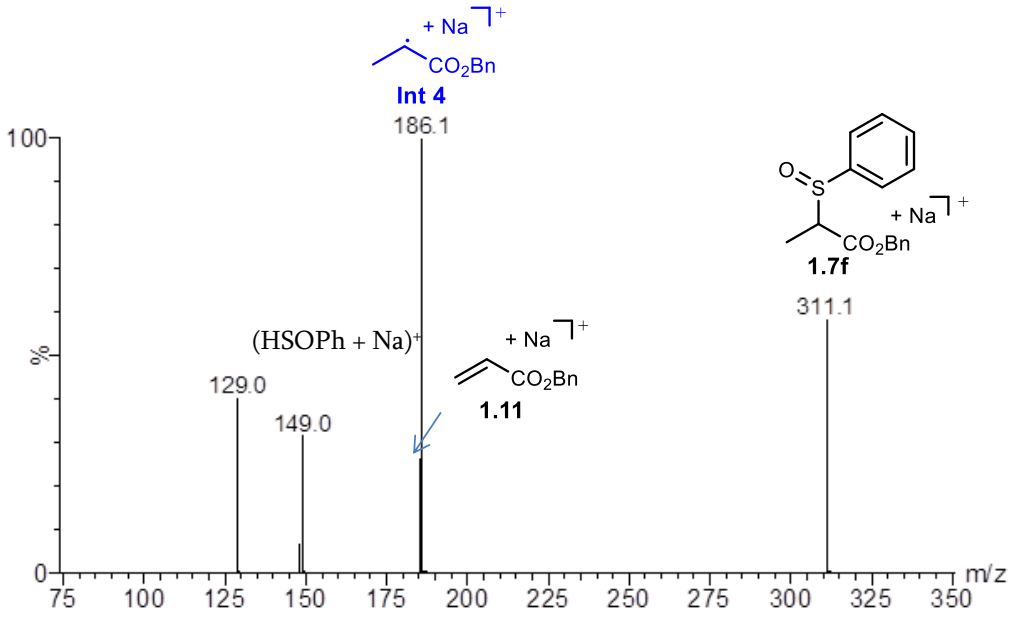



**Figure 13:** Dissociation spectrum of compound **1.7f**

To confirm the same results than in the solution assays, 2-aryl sulfide esters and 2-arylsulfonyl esters were submitted to the fragmentation studies. In the CID spectra of those compounds, no radical intermediates were detected. It is known that sulfones and thioethers do not eliminate as easily as sulfoxides do.[31]

[31] Cubagge, J. W.; Vos, B. W.; Jenks, W. S. *J. Am. Chem. Soc.* **2000**, 122, 4968– 4971

# 3. Conclusions

With these results, a complete reaction pathway was proposed (**scheme 19**) which explains the formation of all detected by-products. It was essential to have a complete view of all possible products before demonstrating the correct pathway.

The presence of diphenyl disulfide **1.25** can be explained from the partially conversion from phenyl-thiosulfonate to diphenyl disulfide **1.25** and benzene sulfonic acid. The addition of sulfenic acids to unsaturated esters has been previously demonstrated,[32,33,34] furthermore the low yield in the formation of rearrangement compounds as **1.13** is due to the high tendency of sulfinyl radicals or sulfenic acids to afford thiosulphonates. This final reaction pathway would depend on the subtle interplay between kinetics and thermodynamics of each elementary step.

It was demonstrated the radical mechanism of dehydrosulfenylation of 2-arylsulfinyl esters to afford enoates. The presence of radicalary intermediates in the mechanism was unequivocally confirmed by trapping them with tempo and by a

[32] Shelton, J. R.; Davis, K. E. *J. Am. Chem. Soc.* **1967**, 89, 718– 719
[33] Barton, D. H. R.; Comer, F.; Greig, D. G. T.; Lucente, G.; Sammes, P. G.; Underwood, W. G. *J. Chem. Soc., Chem. Commun.* **1970**, 17, 1059-1060
[34] Barton, D. H. R.; Sammes, P. G.; Taylor, M. V.; Cooper, C. M.; Hewitt, G.; Looker, B. F.; Underwood, W. G. *J. Chem. Soc., Chem. Commun.* **1971**, 18, 1137-1139

crossover experiment. The ESI-MS and tandem ESI-MS was an essential tool to detect these radical intermediates and their subsequent transformations in a monitored reaction, providing information of the mechanism.

The radical mechanism pathway results to be complementary to the internal elimination (Ei) on literature. According to these previous results and this study, depending on the starting compound and thermodynamics and kinetics intermediate steps will determine the pathway to form the enoates.

An unpreceded transformation of 2-arylsulfinyl esters to 3-arylsulfinyl esters as a side reaction was also observed based on radical mechanism.

# 4. Experimental information

## 4.1. General methods

All solvents used in reactions were freshly distilled from appropriate drying agents before use. $^{1}H$ NMR spectra and $^{13}C$ NMR spectra were measured in $CDCl_3$ ($^{1}H$, 7.24 ppm; $^{13}C$ 77.0 ppm) solution at 30 °C on a 300 MHz or a 500 MHz NMR spectrometer. IR spectra were recorded as oil films or KBr discs or NaCl pellets on a FT-IR spectrometer. Silica gel 60 was used for column chromatography, while TLC was performed with precoated plates (Kieselgel 60, $F_{254}$, 0.25 mm). Unless otherwise specified, all reactions were carried out under argon atmosphere with magnetic stirring.

Electrospray Ionization Mass Spectrometry (ESI–MS) and Electron Impact Mass Spectrometry (EI–MS)

For the online reaction monitoring, ESI mass spectra were recorded using an ESI tandem mass spectrometer (quadrupole-hexapole-quadrupole). The drying gas was nitrogen. The temperature of the source block was set to 120 °C and the desolvation temperature to 150 °C. A capillary voltage of 3.5 kV was used in the positive scan mode, and the cone voltage ($U_c$) was set to 15 V to control the extent of fragmentation of the identified ions. Toluene solutions of **1.7a–f** were heated at 100 °C in an open vial, a drop of the solution was immediately extracted and diluted in hot $CH_3CN$, and a positive-ion mass spectrum was collected. For CID experiments, the cations of interest were mass-selected using the first quadrupole (Q1) and interacted with argon in the hexapole collision cell at variable collision energies (typically in the $E_{laboratory}$ = 3–10 eV range) while mass analyzing the products with the second analyzer. The isolation width was ca. 1 Da and argon was used as a collision gas to produce a pressure of $8 \times 10^{-4}$ mbar.

For accurate $m/z$ determinations (for all the compounds except for diphenyl disulfide **1.25** and CID experiments, an ESI tandem mass spectrometer (quadrupole-Twave-time-of-flight) was used. The drying gas was nitrogen. The temperature of the source block was set to 120 °C and the desolvation temperature to 150 °C. A capillary voltage of 3.5 kV was used in the positive scan mode, and the cone voltage was set to a low value to control the extent of fragmentation (typically 15 V). Methanol sample solutions were infused via syringe pump directly connected to the ESI source at a flow rate of 10 μL/min. Mass calibration was performed using a mixture of NaOH 0.05 M/formic acid 10% (50:50) from $m/z$ 50 to 900. For accurate mass measurements, a solution of leucine enkephalin ($m/z$ = 556.2771) was introduced via the lock spray needle at a flow rate of 30 μL/min.

For an accurate *m*/*z* determination diphenyl disulfide **1.25**, a GC–EIMS was used. The GC instrumentation used was equipped with an autosampler coupled to a TOF mass spectrometer operating in electron ionization (EI) mode. The GC separation was performed using a fused silica column (30 m × 0.25 mm i.d., 0.25 mm film thickness). The oven temperature was programmed as follows: 90 °C (hold 1 min); 10 °C/min to 300 °C (hold 2 min). The total running time was 24 min. Splitless injections of 1 mL of sample extracts were carried out with an injector temperature of 300 °C and with a splitless time of 1 min. Helium 99.999% was used as carrier gas at a constant flow of 1 mL/min. The interface and ion source temperatures were set to 260 and 250 °C, respectively. A solvent delay of 3 min was used to prevent damage in the ion source filament. TOF MS was operated at an scan time of 0.95 s in the mass range *m*/*z* 60–300 and using a multichannel plate voltage of 2700 V. TOF MS resolution was about 8500 (fwhm) at *m*/*z* 614. Pure PFTBA (Perfluorotri-*n*-butylamine), used for the daily mass calibration/verification as well as for lock mass, was injected via syringe (~ 1 mL) in the reference reservoir at 30 °C. The *m*/*z* monitored was 218.9856.

## X-ray Crystallography

Crystals of compound **1.5** are air stable and were mounted on the tip of a glass fiber with the use of epoxi cement. X-ray diffraction experiments were carried out on a diffractometer using Mo Kα radiation (λ = 0.71073 Å) at room temperature. The data were collected with a frame width of 0.3° in ω and a counting time of 60 s per frame at a crystal to detector distance of 4 cm. The diffraction frames were integrated using the SAINT package and corrected for absorption with SADABS. The structures were solved by direct methods and refined by the full-matrix method based on $F^2$ using the SHELXTL software

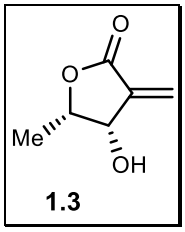
package. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were generated geometrically, assigned isotropic thermal parameters and allowed to ride on their respective parent carbon atoms. Crystal data and structure refinement for **1.5**: empirical formula $C_{12}H_{14}O_5S$; crystal system monoclinic; space group *P2*(1); unit cell dimensions $a$ = 4.8321(7) Å, $b$ = 21.157(3) Å, $c$ = 6.4661(9) Å, β = 95.816(3)°; $Z$ = 2; θ range for data collection 1.93–30.49°; reflections collected 5373; independent reflections 3114 [$R$(int) = 0.0247]; goodness-of-fit on $F^2$ = 1.030; final $R$ indices [$I > 2\sigma(I)$] R1 = 0.0424, wR2 = 0.0928; $R$ indices (all data) R1 = 0.0685, wR2 = 0.1034; absolute structure parameter –0.03(8); largest diff peak and hole 0.268 and –0.180 e·Å$^{-3}$.

## 4.2. General experiment for compound preparation

Preparation (4S,5S)-Dihydro-4-hydroxy-5-methyl-3-methylenefuran-2(3H)-one (**1.3**) and (3R,4S,5S)-Dihydro-4-hydroxy-5-methyl-3-((phenylsulfonyl)methyl)furan-2(3H)-one (**1.5**)

An oxygen-flushed mixture of compound **1.2** (124 mg, 0.48 mmol) in toluene (8 mL) was heated at 110 °C for 5 h. Then the solvent was removed under vacuum and the resulting mixture was purified through chromatography (silica gel, hexanes/ethyl acetate (1:1), (1:2) and ethyl acetate) to afford 44 mg (71%) of compound **1.3** as a colorless oil and 16 mg (12%) of compound **1.5** as a white solid. Recrystallization from $CHCl_3$/hexanes gave white needles (mp = 117–120 °C).

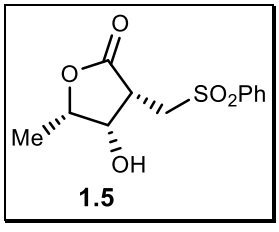
Spectroscopic data of **1.3**:




**1.3**

$[\alpha]_D^{20}$ = –97.26 (*c* = 1.3, $CHCl_3$); $^1H$ NMR (300 MHz, $CDCl_3$) δ 6.41 (1H, d, *J* = 2.1 Hz), 5.97 (1H, d, J=1.5 Hz), 4.83 (1H, d, *J* = 5.7 Hz), 4.65 (1H, dq, *J* = 6.0, 6.6 Hz), 1.34 (3H, d, *J* = 6.6 Hz); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 168.9, 138.8, 126.3, 78.4, 69.7, 14.3; IR (NaCl) δ 3439, 3015, 2930, 1756, 1672, 1387, 1263, 1186, 1103, 1045, 958, 910, 861, 821, 784 $cm^{-1}$; HRMS *m*/*z* calcd for $C_6H_8O_3Na$ [M + $Na^+$] 151.0371, found 151.0354.

Spectroscopic data of **1.5**:



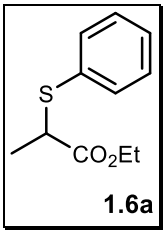

**1.5**

$[\alpha]_D^{26}$ = –33.94 (*c* = 1, $CHCl_3$); $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.95 (2H, d, *J* = 7.2 Hz), 7.72 (t, 1H, *J* = 7.5 Hz), 7.61 (t, 2H, *J* = 7.8 Hz), 4.72 (m, 1H), 4.63 (m, 1H), 3.56 (m, 2H), 3.26 (m, 1H), 2.93 (d, 1H, J = 4.2 Hz), 1.49 (3H, d, *J* = 6.6 Hz); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 174.6, 138.4, 134.5, 129.7, 127.8, 79.6, 70.1, 51.2, 42.7, 13.6 ppm; IR (NaCl) δ 3350, 2949, 2836, 1764, 1656, 1449, 1412, 1117, 1033 $cm^{-1}$; HRMS *m*/*z* calcd for $C_{12}H_{14}O_5SNa$ [M + $Na^+$] 293.0460, found 293.0429.

General Experimental Procedure for the Preparation of Thioethers **1.6a–f**

To an ice-bath solution of sodium hydride (1.85 g, 46.2 mmol) (60% in mineral oil) in tetrahydrofuran (110 mL) was added dropwise the corresponding thiophenol (92.4 mmol). The resulting mixture was stirred in an ice bath for 30 min. The bromoester (92.4 mmol) was added, and the resulting mixture was stirred at room temperature for 72 h. The mixture was quenched with brine and extracted with ethyl ether (3 × 15 mL), and then the organic layers were washed with a saturated aqueous solution of sodium bicarbonate and water, dried ($Na_2SO_4$), and concentrated. The crude was purified through chromatography (silica gel, hexanes/ethyl acetate (95:5)) to afford the desired compound.

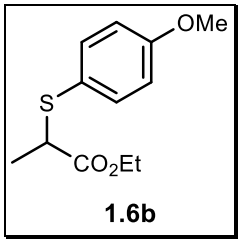
Ethyl 2-(phenylthio)propanoate (**1.6a**):




**1.6a**

yield = 12.61 g, 65%; $^1$H NMR (300 MHz, $CDCl_3$) δ 7.46–7.48 (2H, m), 7.26–7.32 (3H, m), 4.11 (2H, q, *J* = 7.2 Hz), 3.78 (1H, q, *J* = 6.9 Hz), 1.48 (3H, d, *J* = 6.9 Hz), 1.17 (3H, t, *J* = 7.2 Hz); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 173.0, 133.4, 129.2, 128.3, 61.5, 45.6, 17.7, 14.3 ppm; IR (NaCl) δ 3060, 2981, 2933, 2872, 1955, 1883, 1732, 1583, 1476, 1440, 1375, 1323, 1256, 1225, 1159, 1068, 1025, 897, 859, 776, 748, 691, 596 cm$^{-1}$; HRMS *m*/*z* calcd for $C_{11}H_{14}O_2SNa$ [M + $Na^+$] 233.0612, found 233.0611.

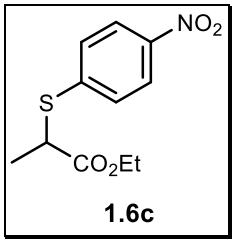
Ethyl 2-((4-methoxyphenyl)thio)propanoate (**1.6b**):




**1.6b**

yield = 13.3 g, 60%; $^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.36 (2H, d, *J* = 8.0 Hz), 6.79 (2H, d, *J* = 8.5 Hz), 4.06 (2H, q, *J* = 7.0 Hz), 3.75 (3H, s), 3.57 (1H, q, *J* = 7.0 Hz), 1.37 (3H, d, *J* = 7.0 Hz), 1.14 (3H, t, *J* = 7.5 Hz); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 172.9, 160.5, 136.7, 123.4, 114.7, 61.2, 55.5, 46.2, 17.4, 14.3 ppm; IR (NaCl) δ 2927, 1727, 1591, 1493, 1461, 1286, 1247, 1172, 1029, 827 $cm^{-1}$; HRMS *m*/*z* calcd for $C_{12}H_{16}O_3SNa$ [M + $Na^+$] 263.0718, found 263.0712.

Ethyl 2-((4-nitrophenyl)thio)propanoate (**1.6c**):

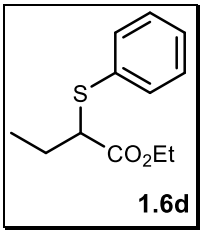



**1.6c**

yield = 12.25 g, 52%; $^{1}$H NMR (500 MHz, $CDCl_3$) δ 8.11 (2H, d, *J* = 8.5 Hz), 7.46 (2H, d, *J* = 9.0 Hz), 4.15 (2H, m), 4.00 (2H, q, *J* = 7.0 Hz), 1.56 (3H, d, *J* = 7.5 Hz), 1.19 (3H, t, *J* = 7.0 Hz); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 172.2, 144.9, 131.5, 129.3, 124.3, 62.1, 44.1, 17.6, 14.4 ppm; IR (NaCl) δ 2925, 1732, 1596, 1578, 1515, 1477,

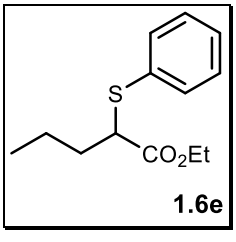
1341, 1259, 1174, 1076, 1013, 853, 742, 683 $cm^{-1}$; HRMS *m/z* calcd for $C_{11}H_{14}NO_4S$ [M + $H^+$] 256.0644, found 256.0647; calcd for $C_{11}H_{13}NO_4SNa$ [M + $Na^+$] 278.0463, found 278.0464.

Ethyl 2-(phenylthio)butanoate (**1.6d**):




yield = 19.25 g, 93%; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.44 (2H, m), 7.25 (3H, m), 4.09 (2H, dq, *J* = 1.2, 7.2 Hz), 3.56 (1H, dd, *J* = 6.6, 8.4 Hz), 1.70–1.95 (m, 2H), 1.15 (3H, t, *J* = 6.9 Hz), 1.09 (3H, t, *J* = 7.2 Hz); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 172.0, 133.7, 132.7, 128.8, 127.7, 60.9, 52.4, 25.1, 14.0, 11.7 ppm; IR (oil) δ 3079, 2970, 2936, 2877, 1959, 1884, 1735, 1583, 1480, 1443, 1368, 1343, 1300, 1234, 1209, 1160, 1091, 1026, 941, 864, 811 $cm^{-1}$; HRMS *m/z* calcd for $C_{12}H_{17}O_2S$ [M + $H^+$] 225.0949, found 225.0953; calcd for $C_{12}H_{16}O_2SNa$ [M + $Na^+$] 247.0769, found 247.0772.

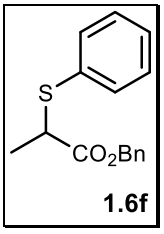
Ethyl 2-(phenylthio)pentanoate (**1.6e**):




yield = 19.35 g, 88%; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.43–7.47 (2H, m), 7.24–7.31 (3H, m), 4.10 (2H, q, *J* = 7.2 Hz), 3.65 (1H, dd, *J* = 6.6, 8.1 Hz), 1.67–1.94 (m, 2H),

1.38–1.52 (m, 2H), 1.15 (3H, t, $J$ = 6.6 Hz), 0.92 (3H, t, $J$ = 7.2 Hz); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 172.0, 133.7, 132.7, 128.7, 127.6, 60.7, 50.5, 33.7, 20.5, 14.0, 13.6 ppm; IR (oil) δ 3066, 2968, 2936, 2876, 1958, 1884, 1733, 1585, 1483, 1444, 1374, 1335, 1303, 1279, 1243, 1163, 1106, 1033, 934, 860, 808 $cm^{-1}$; HRMS $m/z$ calcd for $C_{13}H_{19}O_2S$ [M + $H^+$] 239.1106, found 239.1115; calcd for $C_{13}H_{18}O_2SNa$ [M + $Na^+$] 261.0925, found 261.0923.

Benzyl 2-(phenylthio)propanoate (**1.6f**):



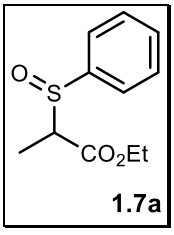

yield = 17.59 g, 70%; $^1$H NMR (300 MHz, $CDCl_3$) δ 7.24–7.44 (10H, m), 5.11 (2H, s), 3.85 (1H, d, $J$ = 7.2 Hz), 1.52 (3H, d, $J$ = 7.2 Hz); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 172.5, 135.5, 133.2, 133.0, 128.9, 128.5, 128.2, 128.0, 66.7, 45.3, 17.3 ppm; IR (oil) δ 3061, 2986, 2936, 1959, 1888, 1735, 1586, 1480, 1443, 1380, 1324, 1268, 1162, 1063, 1029, 1004, 954, 898, 780 $cm^{-1}$; HRMS $m/z$ calcd for $C_{16}H_{17}O_2S$ [M + $H^+$] 273.0949, found 273.0946; calcd for $C_{16}H_{16}O_2SNa$ [M + $Na^+$] 295.0769, found 295.0770.

## General Experimental Procedure for the Preparation of Sulfoxides **1.7a–f**

To a –10 °C cold solution of thioether (0.95 mmol) in $CH_2Cl_2$ (5 mL) was added a solution of *m*-CPBA (77% pure) (177 mg, 0.79 mmol) in $CH_2Cl_2$ (4 mL). The resulting mixture was stirred at –10 °C for 30 min, quenched with saturated

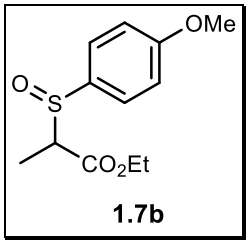
aqueous solution of sodium bicarbonate, and extracted with $CH_2Cl_2$ (3 × 15 mL), and then the organic layers were sequentially washed with brine and saturated aqueous solution of sodium bicarbonate and water, dried ($Na_2SO_4$), and concentrated. The crude was purified through chromatography (silica gel, hexanes/ethyl acetate (7:3)) to afford the desired sulfoxide.

Ethyl 2-(phenylsulfinyl)propanoate (**1.7a**):




yield = 178 mg, 83%); $^1$H NMR (500 MHz, $CDCl_3$) δ 7.25–7.45 (5H, m) (major and minor), 4.12 (2H, m) (major and minor), 3.81 (1H, q, $J$ = 6.5 Hz) (major), 3.49 (1H, q, $J$ = 7.0 Hz) (minor), 1.49 (3H, d, $J$ = 7.0 Hz) (major), 1.32 (3H, d, $J$ = 7.5 Hz) (minor), 1.21 (3H, t, $J$ = 6.5 Hz) (minor), 1.18 (3H, t, $J$ = 7.0 Hz) (major); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 168.9, 168.1, 142.6, 140.9, 134.5, 132.1, 131.9, 129.7, 129.5, 129.3, 125.5, 125.1, 66.2, 64.1, 62.1, 62.0, 14.3, 14.2, 10.0, 9.1 ppm; IR (NaCl) δ 3051, 2988, 2926, 1968, 1895, 1724, 1577, 1472, 1445, 1371, 1316, 1254, 1215, 1165, 1083, 1045, 1017, 920, 889, 858, 749, 691 cm$^{-1}$; HRMS $m/z$ calcd for $C_{11}H_{14}O_3SNa$ [M + Na$^+$] 249.0556, found 249.0557.

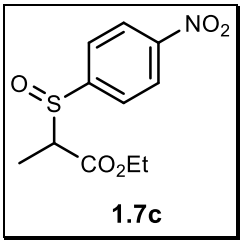
Ethyl 2-((4-methoxyphenyl)sulfinyl)propanoate (**1.7b**):




**1.7b**

yield = 124 mg, 51%; $^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.56 (2H, d, *J* = 8.5 Hz) (major), 7.52 (2H, d,*J* = 8.5 Hz) (minor), 7.00 (2H, d, *J* = 8.5 Hz) (major and minor), 4.04–4.20 (2H, m) (major and minor), 3.85 (3H, s) (major and minor), 3.78 (1H, q, *J* = 7.0 Hz) (major), 3.47 (1H, q, *J* = 8.0 Hz) (minor), 1.50 (3H, d, *J* = 7.0 Hz) (major), 1.27 (3H, d, *J* = 7.0 Hz) (minor), 1.23 (3H, t, *J* = 7.0 Hz) (minor), 1.15 (3H, t, *J* = 7.0 Hz) (major); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 169.1, 168.3, 162.7, 133.2, 131.5, 127.3, 126.9, 114.9, 114.8, 66.3, 64.3, 61.9, 55.8, 14.3, 14.3, 10.4, 8.9 ppm; IR (KBr) δ 3096, 3069, 2981, 2940, 2842, 1727, 1598, 1500, 1459, 1306, 1258, 1173, 1092, 1024, 834, 793, 626 $cm^{-1}$; HRMS *m/z* calcd for $C_{12}H_{17}O_4S$ [M + $H^+$] 257.0848, found 257.0849; calcd for $C_{12}H_{16}O_4SNa$ [M + $Na^+$] 279.0667, found 279.0656.

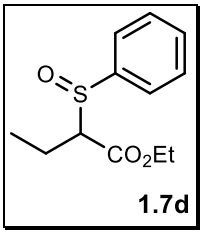
Ethyl 2-((4-nitrophenyl)sulfinyl)propanoate (**1.7c**):




**1.7c**

yield = 160 mg, 62%; $^{1}$H NMR (500 MHz, $CDCl_3$) δ 8.31 (2H, d, *J* = 8.5 Hz) (major), 8.30 (2H, d,*J* = 9.0 Hz) (minor), 7.82 (2H, d, *J* = 9.0 Hz) (major), 7.77 (2H, d, *J* = 9.0 Hz) (minor), 4.04–4.15 (2H, m) (major and minor), 3.84 (1H, q, *J* = 7.0 Hz) (major), 3.48 (1H, q, *J* = 7.0 Hz) (minor), 1.41 (3H, d, *J* = 7.5 Hz) (major), 1.30 (3H, d, *J* = 7.0 Hz) (minor), 1.17 (3H, t, *J* = 7.5 Hz) (minor), 1.15 (3H, t, *J* = 7.5 Hz) (major); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 169.6, 168.3, 149.7, 147.9, 126.6, 126.2, 127.3, 125.6, 125.0, 65.6, 63.7, 62.5, 62.3, 14.2, 9.4, 9.0 ppm; IR (NaCl) δ 3101, 2985, 1732, 1603, 1579, 1527, 1475, 1450, 1398, 1347, 1150, 1089, 1054, 1011, 1017, 853, 743, 723, 684 $cm^{-1}$; HRMS *m*/*z* calcd for $C_{11}H_{14}NO_5S$ [M + $H^+$] 272.0593, found 272.0580; calcd for $C_{11}H_{13}NO_5SNa$ [M + $Na^+$] 294.0412, found 294.0405.

Ethyl 2-(phenylsulfinyl)butanoate (**1.7d**):

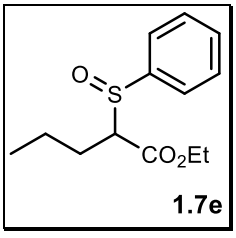



**1.7d**

yield = 187 mg, 82%; $^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.49–7.66 (5H, m) (major and minor), 3.95–4.10 (2H, m) (major and minor), 3.48 (1H, dd, *J* = 5.0, 10.0 Hz)

(major), 3.39 (1H, dd, $J$ = 5.0, 9.5 Hz) (minor), 2.06–2.16 (1H, m) (major and minor), 1.76–1.90 (1H, m) (major and minor), 1.12 (3H, t, $J$ = 7.5 Hz) (major), 1.06 (3H, t, $J$ = 7.5 Hz) (minor), 1.03 (3H, t, $J$ = 7.0 Hz) (major), 1.00 (3H, t, $J$ = 7.5 Hz) (minor); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 167.6, 166.7, 142.2, 141.1, 131.7, 131.4, 129.9, 128.9, 124.8, 124.7, 73.5, 70.6, 61.3, 20.3, 18.7, 13.8, 11.5, 11.3 ppm; IR (oil) δ 3066, 2975, 2940, 2880, 2088, 1972, 1894, 1726, 1638, 1581, 1476, 1448, 1374, 1335, 1258, 1202, 1163, 1089, 1039, 952, 860, 815 cm$^{-1}$; HRMS $m/z$ calcd for $C_{12}H_{17}O_3S$ [M + H$^+$] 241.0898, found 241.0900; calcd for $C_{12}H_{16}O_3SNa$ [M + Na$^+$] 263.0718, found 263.0722.

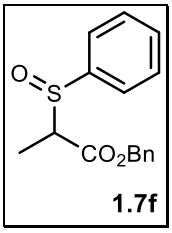
Ethyl 2-(phenylsulfinyl)pentanoate (**1.7e**):




yield = 222 mg, 92%; $^1$H NMR (500 MHz, $CDCl_3$) δ 7.46–7.65 (5H, m) (major and minor), 3.88–4.10 (2H, m) (major and minor), 3.53 (1H, dd, $J$ = 5.5, 10.0 Hz) (major), 3.43 (1H, dd, $J$ = 5.0, 9.0 Hz) (minor), 2.02–2.10 (1H, m) (major and minor), 1.73–1.84 (1H, m) (major and minor), 1.28–1.48 (2H, m) (major and minor), 1.09 (3H, t, $J$ = 7.0 Hz) (major), 1.03 (3H, t, $J$ = 7.5 Hz) (minor), 0.91 (3H, t, $J$ = 7.0 Hz) (major), 0.88 (3H, t, $J$ = 7.5 Hz) (minor); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 167.9, 166.8, 142.3, 141.7, 131.7, 131.5, 129.0, 128.8, 124.8, 124.7, 72.1, 68.8, 61.2, 28.7, 27.0, 20.3, 13.8, 13.5 ppm; IR (oil) δ 3062, 2968, 2940, 2879, 2095, 1972, 1891, 1729, 1585, 1469, 1447, 1377, 1335, 1275, 1247, 1191, 1156, 1089, 1057, 941, 860,

825 $cm^{-1}$; HRMS *m/z* calcd for $C_{13}H_{19}O_3S$ [M + $H^+$] 255.1055, found 255.1053; calcd for $C_{13}H_{18}O_3SNa$ [M + $Na^+$] 277.0874, found 277.0876.

Benzyl 2-(phenylsulfinyl)propanoate **(1.7f)**:



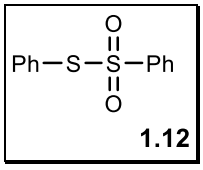

yield = 192 mg, 70%; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.17–7.55 (10H, m) (major and minor), 5.10 (1H, d, *J* = 12.0 Hz) (major), 5.10 (1H, d, *J* = 12.0 Hz) (minor), 5.00 (1H, d, *J* = 12.0 Hz) (major), 4.99 (1H, d, *J* = 12.0 Hz) (minor), 3.82 (1H, q, *J* = 7.2 Hz) (major), 3.50 (1H, q, *J* = 7.2 Hz) (minor), 1.41 (3H, d, *J* = 6.9 Hz) (major), 1.26 (3H, d, *J* = 7.2 Hz) (minor); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 168.4, 167.5, 142.0, 140.2, 134.9, 131.7, 131.5, 129.1, 128.9, 128.6, 128.5, 128.4, 128.3, 125.0, 124.6, 67.4, 67.3, 65.4, 63.5, 9.4, 8.6 ppm; IR (oil) δ 3067, 3036, 2939, 1965, 1894, 1732, 1583, 1499, 1477, 1449, 1380, 1318, 1222, 1163, 1050, 998, 957, 920, 848, 820, 777 $cm^{-1}$; HRMS *m/z* calcd for $C_{16}H_{17}O_3S$ [M + $H^+$] 289.0898, found 289.0894; calcd for $C_{16}H_{16}O_3SNa$ [M + $Na^+$] 311.0718, found 311.0713.

## Experimental Procedure for Elimination

A mixture of sulfinyl compound **1.7** (1 mmol) in toluene (6 mL) was heated at 110 °C for 5 h (in the case of compounds **1.7a**, **1.7b**, **1.7c** and **1.7f**, the reaction was performed in a sealed flask to avoid acrylate loss). Then the resulting crude

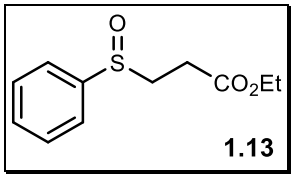
mixture was purified through chromatography (silica gel, hexanes/ethyl acetate (1:1), (1:2) and ethyl acetate) to afford the corresponding enoate.

*S*-phenyl benzenesulfonothioate **(1.12):**



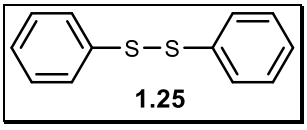

$^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.13–7.51 (10H, m); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 143.0, 136.6, 133.6, 131.4, 129.4, 129.0, 128.8, 127.9, 127.6, 127.2 ppm; IR (NaCl) δ 3064, 1580, 1445, 1323, 1144, 1076, 1021, 748, 688 $cm^{-1}$; HRMS *m*/*z* calcd for $C_{12}H_{10}O_2S_2Na$ [M + $Na^+$] 273.0020, found 273.0026.

2-(Ethoxycarbonyl)ethyl benzenesulfenate **(1.13):**




$^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.61–7.62 (2H, m), 7.49–7.54 (3H, m), 4.11 (2H, q, *J* = 7.5 Hz), 3.22 (1H, m), 2.97 (1H, m), 2.84 (1H, m), 2.54 (1H, m), 1.22 (3H, d, *J* = 7.5 Hz); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 171.5, 143.4, 131.5, 129.6, 124.4, 61.4, 51.5, 26.5, 14.4 ppm; IR (NaCl) δ 2963, 1733, 1445, 1373, 1260, 1087, 1045, 799, 750, 691 $cm^{-1}$; HRMS *m*/*z* calcd for $C_{11}H_{14}O_3SNa$ [M + $Na^+$] 249.0561, found 249.0561.

Diphenyl disulfide (**1.25**):



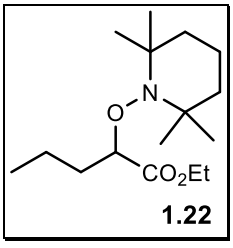

**1.25**

$^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.13–7.17 (2H, m), 7.21–7.24 (4H, t, J = 8.5 Hz), 7.42 (4H, d, J = 8.5 Hz); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 137.1, 129.0, 127.6, 127.2 ppm; IR (NaCl) δ 3064, 1946, 1864, 1800, 1735, 1574, 1470, 1432, 1379, 1298, 1068, 1017, 896, 787, 735, 686, 605 $cm^{-1}$; HRMS (EI) *m/z* calcd for $C_{12}H_{10}S_2$ [M] 218.0224, found 218.0210.

Experimental Procedure for Trapping the Radical

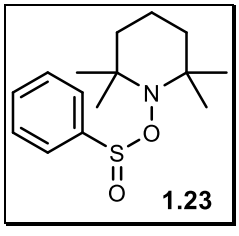
A mixture of sulfinyl compound **1.7e** (500 mg, 1.96 mmol) and 2,2,6,6-Tetramethyl-1-piperidinyloxy (Tempo) (614 mg, 3.93 mmol) in toluene (10 mL) was heated at 80 °C for 2 h. The resulting crude mixture was carefully concentrated under vacuum and then purified through chromatography (silica gel, hexanes/ethyl acetate (95:5)) to afford compound **1.22** (17 mg, 3%) as an oil and compound **1.23** (60 mg, 11%) as a white solid. Recrystallization of **1.23** from $CH_2Cl_2$/hexanes gave white prismatic crystals (mp =128–130 °C).

Ethyl 2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)pentanoate **(1.22):**




$^{1}$H NMR (300 MHz, $CDCl_3$) δ 4.21 (1H, dd, *J* = 6.6, 7.5 Hz), 4.15 (2H, q, *J* = 7.2 Hz), 1.74–1.85 (2H, m), 1.01–1.48 (23H, m), 0.91 (3H, t, *J* = 7.2 Hz); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 173.6, 85.5, 60.1, 40.3, 34.2, 33.6, 33.0, 20.2, 17.9, 17.1, 14.2, 13.9 ppm; IR (KBr) δ 2974, 2933, 2876, 1748, 1466, 1381, 1366, 1269, 1245, 1184, 1133, 1105, 1058, 1031, 993, 976, 963, 929, 881, 857, 793, 752, 722, 681, 650 $cm^{-1}$; HRMS *m/z* calcd for $C_{16}H_{32}NO_3$ [M + $H^+$] 286.2382, found 286.2377.

2,2,6,6-Tetramethylpiperidin-1-yl benzenesulfinate **(1.23):**




$^{1}$H NMR (300 MHz, $CDCl_3$) δ 7.82–7.89 (2H, m), 7.39–7.47 (3H, m), 1.66 (6H, s), 1.57 (12H, s);$^{13}$C NMR (75 MHz, $CDCl_3$) δ 147.2, 131.2, 128.5, 126.0, 60.8, 43.8, 31.1, 16.7 ppm; IR (KBr) δ 3069, 3035, 2984, 2944, 2916, 2879, 1404, 1394, 1245, 1204, 1184, 1139, 1095, 1071, 980, 922, 786, 766, 715, 701, 626, 613 $cm^{-1}$; HRMS *m/z* calcd for $C_{15}H_{23}NO_2SNa$ [M + $Na^+$] 304.1347, found 304.1348.

# Chapter 2:

# Study of the stereoisomerization of α-hydroxy-β-sulfenyl-α,β-dimethyl naphthoquinones

# 1. Introduction

Intramolecular nonbonded S···X interactions have been extensively studied for some organic sulfur compounds in relation to the biological activities as well as the physical properties as advanced materials.[35]

For example, it was reported thiazole nucleoside tiazofurin with antitumor activity to adopt an active conformation displaying a 1,4-type S-O interaction.[36] The interaction was postulated based on X-rays crystallographic (sulfur–oxygen distances were less than the sum of the Van der Waals radii (3.3 Å)) and computational studies (**figure 1**).

[35] Iwaoka, M.; Isozuni, M. *Molecules* **2012**, *17*, 7266-7283
[36] Burling, F.T.; Goldstein, B.M. *J. Am. Chem. Soc.* ***1992***, 114, 2313–2320

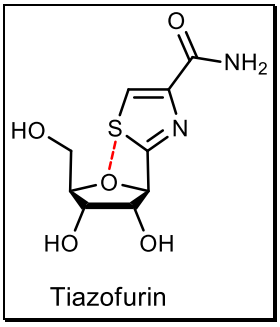



**Figure 1.** Interaction proposed by Goldstein

Nagao reported an 1,5-type S-O interaction in (acylimino)thiadiazoline derivatives, showed in **figure 2**, as angiotensin II receptor antagonists. It was demonstrated by X-ray crystallographic analysis and MO calculations.[37]

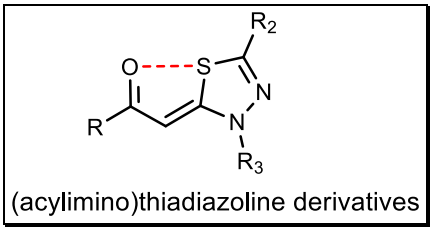



**Figure 2.** Interaction proposed by Nagao

Canadell synthetized an ethylenedithio-tetrathiafulvalene-thiomethyl-oxazoline (EDT-TTF- (SMe)-OX, **figure 3**) derivatives and after theoretical calculations at DFT/B3LYP level and an X-ray crystallographic data analysis, the distance was significantly shorter than the sum of the van der Waals radii again.[38]

[37] Nagao, Y.; Hirata, T.; Goto, S.; Sano, S.; Kakehi, A.; Iizuka, K.; Shiro, M. *J. Am. Chem. Soc.* **1998**, 120, 3104–3110
[38] Réthoré, C.; Madalan, A.; Fourmigué, M.; Canadell, E.; Lopes, E.B.; Almeida, M,; Clérac, R.; Avarvari, N. *New J. Chem.* **2007**, 31, 1468–1483

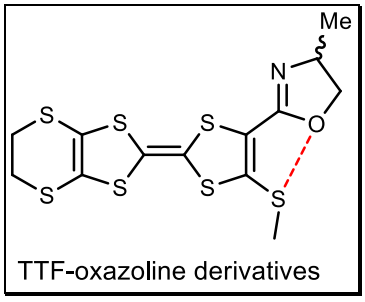



**Figure 3.** Interaction proposed by Canadell

Omelchenko reported based on X-rays diffraction study and quantum-chemical calculations, an intramolecular interaction S-O in thioindirubin (**figure 4**).[39] Again a special short distance between S and O denoted the interaction.

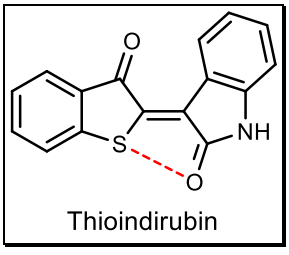



**Figure 4.** Interaction proposed by Omelchenko

In our group, we reported the stereoisomerization of β-hydroxy-α-sulfenyl-γ-butyrolactones controlled by an 1,4-type of S-O interaction. Nine butyrolactones were prepared. It was observed that the *syn-anti* or *syn-syn* lactones (or any mixture of both) invariably isomerized into *syn-anti/syn-syn* lactones in a ratio of 6/4. The other two possible isomeric lactones (*anti-syn* or *anti-anti*) were never observed (**figure 5**).[40]

[39] Shishkin, O.V.; Omelchenko, I.V.; Kalyuzhny, A.L.; Paponov, B.V. *Struct. Chem.* **2010**, 21, 1005–1011
[40] González, F. V.; Jain, A.; Rodríguez, S.; Sáez, J.; Vicent, C.; Peris, G. *J. Org. Chem.* **2010**, 75, 5888-5894

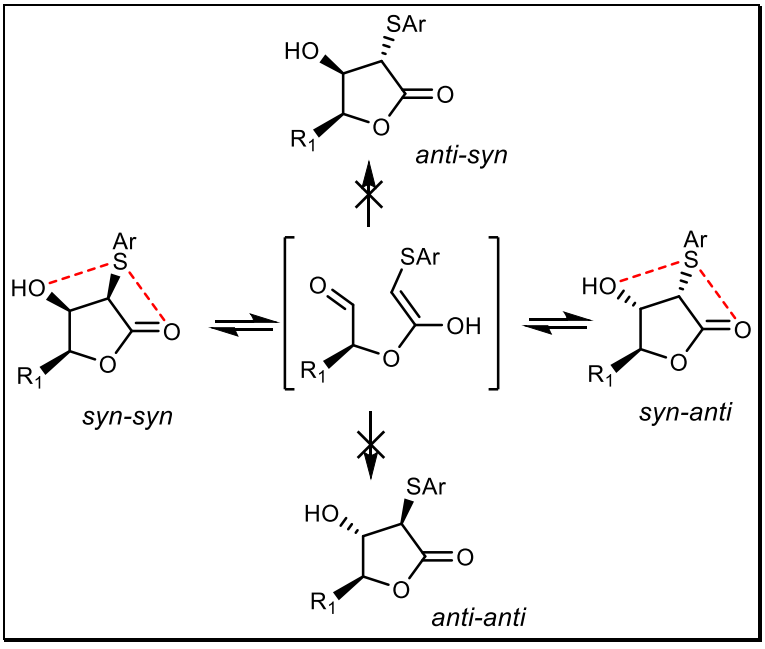



**Figure 5.** Stereoisomerization of β-hydroxy-α-sulfenyl-γ-butyrolactones through a retroaldol-aldol mechanism

According to Parthasarathy,[41] there are two recognizable types of sulfur-oxygen interactions (defined by polar coordinates (polar θ and azimuthal φ angles)): nucleophilic oxygens tend to approach along the extension of one of the covalent bonds to sulfur ($60^{o} < \theta < 90^{o}$) ($110^{o} < \varphi < 150^{o}$) and electrophilic oxygens tend to approach sulfur from the perpendicular direction to the plane through the C-S-C atoms ($\theta < 40^{o}$) (**figure 6**).

[41] Rosenfield, R. E., Jr.; Partharasathy, R.; Dunitz, J. D. *J. Am. Chem. Soc.* **1977**, 99, 4860-4862

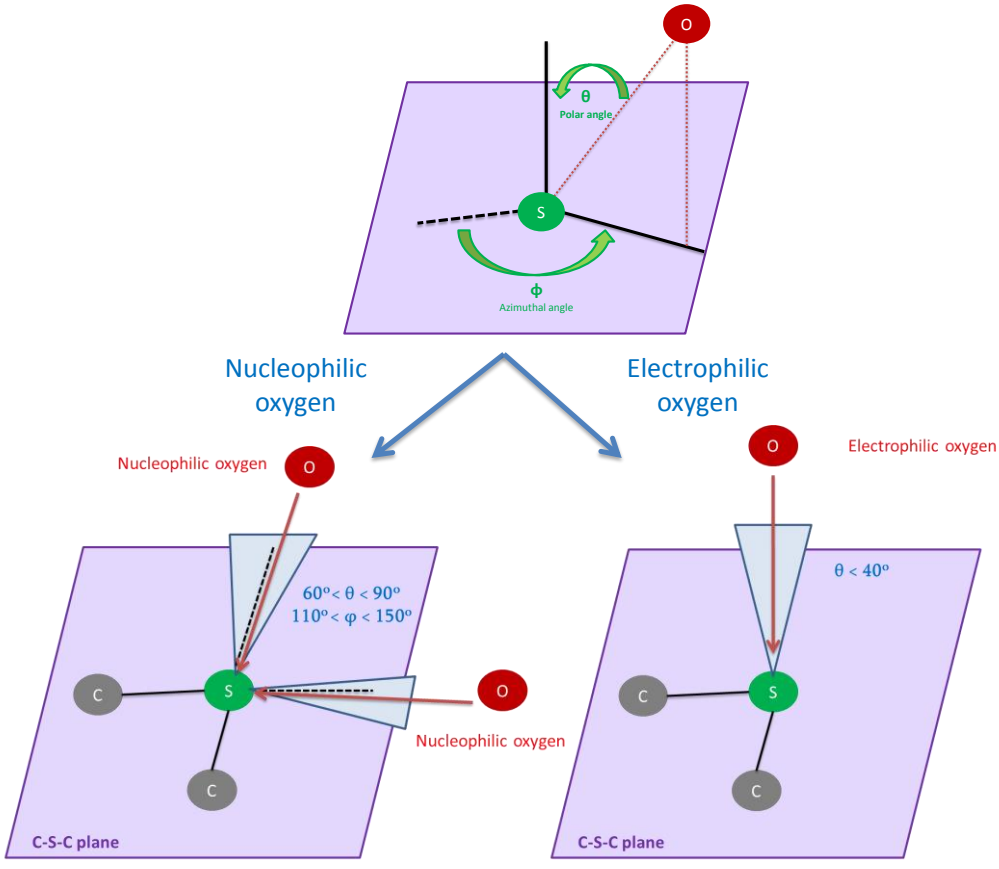



**Figure 6**. Polar coordinates for S-O direction

Crystal structures of *syn–syn* lactones were obtained, and it was observed that the sulfur–oxygen distances were less than the sum of the Van der Waals radii (3.3 Å), demonstrating an interaction. The angle formed by the hydroxyl oxygen, sulfur, and quaternary aromatic carbon being approximately 180° and the carboxylic oxygen–sulfur was directed <40° from the perpendicular to the C–S–C.

It was concluded that two concomitant, attractive 1,4 intramolecular interactions of divalent sulfur with both the carbonyl and the hydroxyl oxygens as

the driving force to establish the stereochemical preference. It was also confirmed that a retroaldol-aldol mechanism operates.

# 2. Results and discussion

The mechanism in the catalytic conversion of vitamin K 2,3-epoxy into vitamin K (**figure 7**), which is essential for blood coagulation, was studied by Silverman.[42,43] Silverman's study was done using 2,3-dimethyl-1,4-naphthoquinone 2,3-epoxide as a model compound.

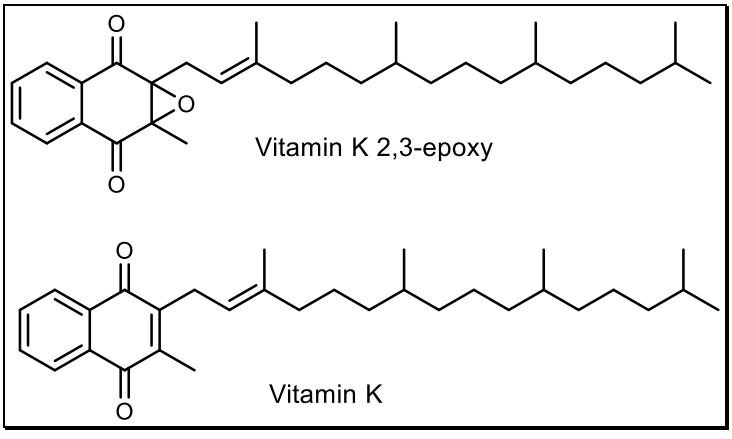
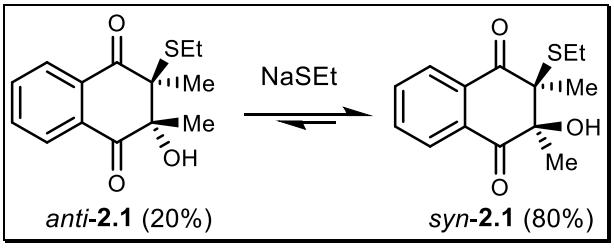



**Figure 7**. Essential vitamin K structures

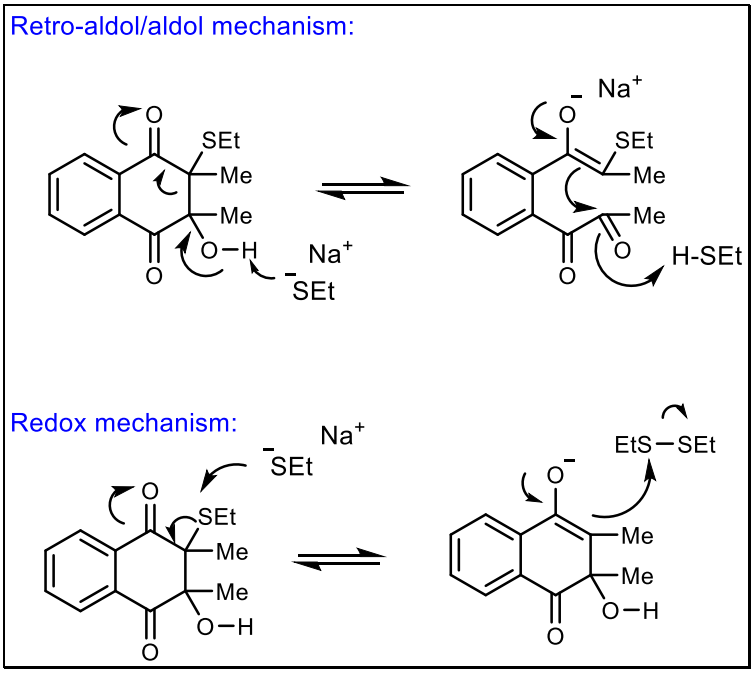
[42] Silverman, R. B. *J. Am. Chem. Soc.* **1981**, 103, 5939-5941
[43] Silverman, R. B. *J. Org. Chem.* **1981**, 46, 4789-4791

When *anti* α-hydroxy-β-ethylsulfenyl-α,β-dimethyl naphthoquinone (anti-**2.1**) was treated with NaSEt a 8:2 mixture of *syn* / *anti* isomers was obtained (**scheme 1**).




**Scheme 1**. Isomerization of Silverman model

Two possible mechanims were proposed: a retro-aldol/aldol mechanism or a redox mechanism (**scheme 2**).




**Scheme 2**. Isomerization mechanisms proposed by Silverman

After this work the mechanism for the natural process of vitamin K was proposed based on the model.

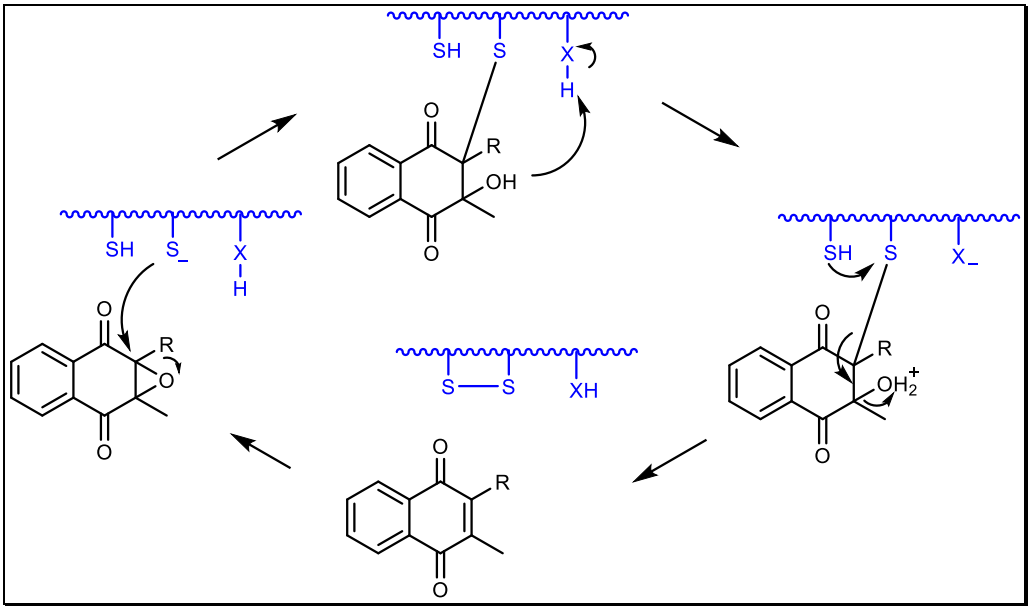
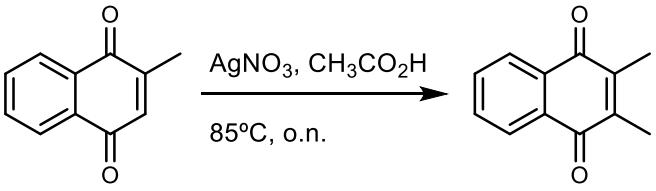


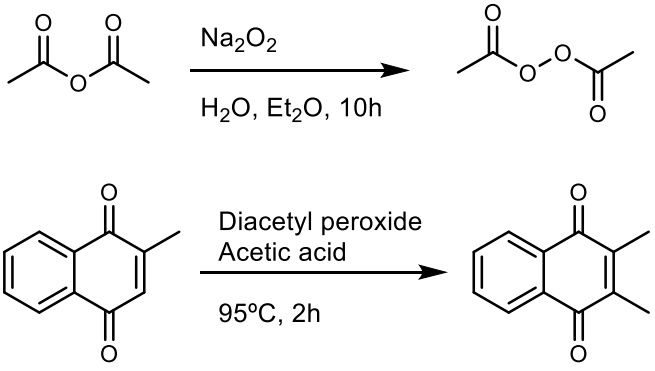

**Figure 8**. Natural process of vitamin K

We decided to explore this reaction. The similarities between Silverman's compounds and previous lactones studied by us (**scheme 1**) prompted us to confirm if sulfur-oxygen interaction might be also operating in this case.

The preparation of the epoxyde model began with the preparation of the 2,3-dimethylnaphthoquinone from menadione. It was prepared by two pathways as described in next **schemes 3** and **4**.

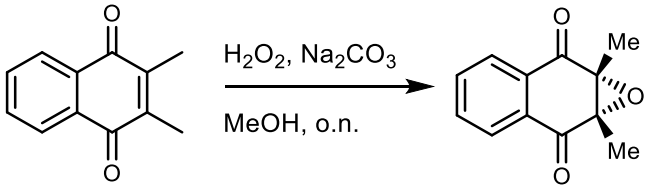



**Scheme 3**. Quinone preparation with first conditions




**Scheme 4**. Quinone preparation with second conditions

The epoxidation was carried out with hydrogen peroxide and $Na_2CO_3$ in MeOH affording the product in excellent yield (95%).

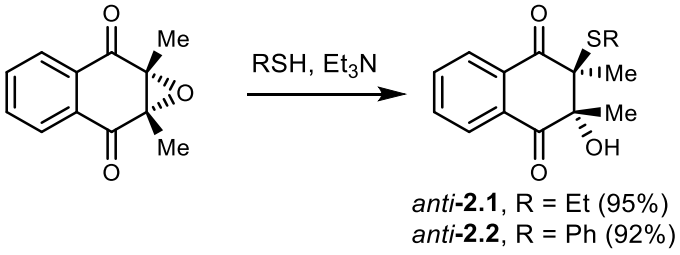



**Scheme 5**. Epoxidation reaction of quinones

Next step was the synthesis of α-hydroxy-β-ethylsulfenyl-α,β-dimethyl naphthoquinone *anti*-**2.1** and *anti*-**2.2** prepared from epoxide.[44] The transformation

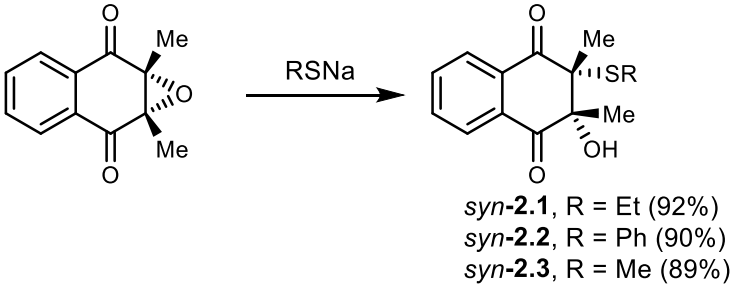
[44] Fieser, L. F.; Campbell,W. P.; Fry, E. M.; Gates, M. D., Jr. *J. Am. Chem. Soc.* **1939**, 61, 3216-3223

was performed as previously described in the literature using the corresponding thiol and triethylamine (**scheme 6**).[8]




**Scheme 6**: Preparation of *anti* naphthoquinones

Naphthoquinones *syn*-**2.1**, *syn*-**2.2** and *syn*-**2.3** were prepared from the epoxide with the adequate sodium thiolate as summarized in **scheme 7**.




**Scheme 7**: Preparation of *syn* naphthoquinones

Firstly we used Silverman's conditions for the isomerization reaction (NaSEt as base) (**table 1**), treating compounds **2.1**-**2.3** with the corresponding sodium ethylthiolate or sodium phenylthiolate, same results as the ones reported by Silverman were obtained. When compound **2.3**, not reported by Silverman, was submitted to same conditions then similar results were also obtained. In all cases *syn* isomers **2.1**-**2.3** were used as a single isomer. When *anti* **2.1**-**2.3** or any mixture of *syn*/*anti* isomers was used as starting materials under reported isomerization

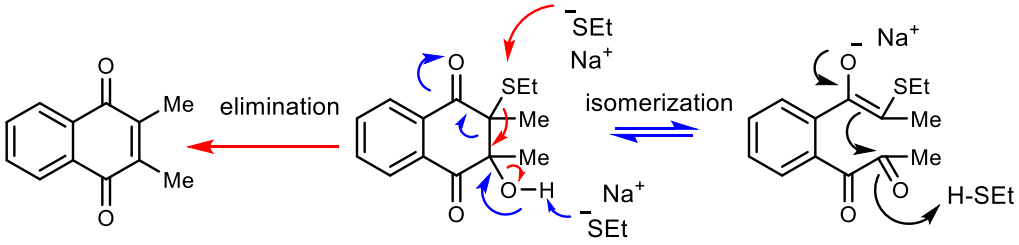
conditions, *syn* isomer was obtained. Compounds **2.1**-**2.3** did not isomerize when triethylamine or N-methyl morpholine are used as bases.

It was remarkable that in some cases (entries 1, 2, 3, 5, 6, 7 and 8) 2,3-dimethylnaphthoquinone as an elimination product was also detected (**scheme 4**). The elimination would result from attack of thiolate to the sulfur atom, to form the corresponding naphthoquinone and the disulfide.

**Table 1. Isomerization reaction**

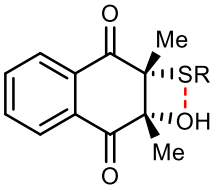
| Entry | Substrate | Base | *anti* / *syn* |
|---|---|---|---|
| 1 | *syn* **2.1** | NaSEt | 14/65[a] |
| 2 | *anti* **2.1** | NaSEt | 14/71[a] |
| 3 | *syn* **2.1** | NaSPh | 5/83[a] |
| 4 | *syn* **2.2** | NaSEt | 17/83 |
| 5 | *syn* **2.2** | NaSPh | 13/61[a] |
| 6 | *anti* **2.2** | NaSEt | 27/68[a] |
| 7 | *anti* **2.2** | NaSPh | 28/69[a] |
| 8 | *syn* **2.3** | NaSEt | 8/82[a] |
| 9 | *syn* **2.3** | NaSPh | 20/80 |
| 10 | *syn* **2.1** | $Et_3N$ | - |
| 11 | *anti* **2.1** | $Et_3N$ | - |
| 12 | *syn* **2.1** | NMM | - |
| 13 | *anti* **2.1** | NMM | - |

[a] Elimination product was detected by NMR




**Scheme 4**: Competing retroaldol-aldol and elimination mechanisms.

We hypothesized *syn* stereochemical preference during isomerization to be controlled by a S-O interaction (**scheme 5**).

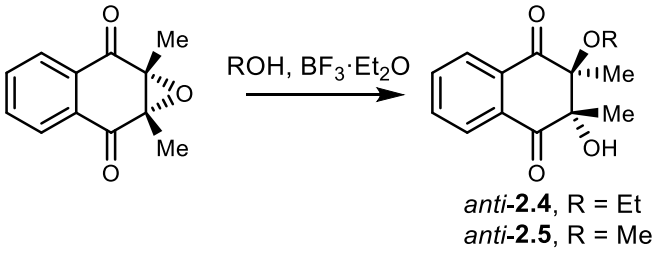



*syn*-**2.1**, R = Et
*syn*-**2.2**, R = Ph
*syn*-**2.3**, R = Me

**Scheme 5**: Proposed S-O interaction

Oxygenated compound **2.4**-**2.5** were prepared to determine the influence of the sulfur atom during isomerization and indirect probe of this interaction. The compounds were prepared starting from 2,3-dimethylnaphthoquinone epoxide using boron trifluoride as catalyst[45] and the corresponding alcohol (ethanol and methanol) (**scheme 6**). This reaction did not work when using phenol. The opening reactions by using alcohols were much slower than when using thiols, curiously same happens in nature since vitamin K epoxide is opened very selectively by cysteine residue (reductase) but not by serine (coagulation factors).[46]

[45] Izquierdo, J.; Rodríguez, S.; González, F. V. *Org. Lett.* **2011**, 13, 3856-3859
[46] Furie, B.; Furie, B. C. *Blood* **1990**, 75, 1753-1762




**Scheme 6**: Preparation of oxygenated *anti* compounds **2.4** and **2.5**

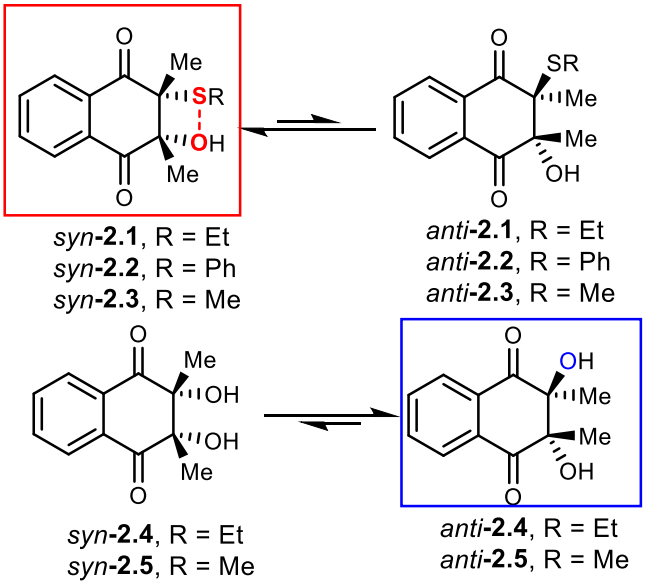
When compounds *anti* **2.4**-**2.5** were submitted to the isomerization conditions using NaSEt as base, a mixture of isomers was obtained. Results from oxygenated compounds **2.4-2.5** were analyzed and compared with sulfurated ones **2.1-2.3**. Compound *anti*-**2.4** was treated with sodium phenylthiolate and sodium ethylthiolate (entries 1-2, **table 2**) affording a mixture of isomers in favor of *anti*-isomer. This result was compared with sulfurated isomerization experiments (entries 1-3, **table 1**) and the opposite tendency was observed.

When compound **2.5** was submitted to isomerization (entries 3-4, **table 2**) a quasi-equal ratio of *syn*/*anti* isomers was detected, while for the sulfur compounds (entries 8-9, **table 1**) *syn* isomer was the major one in all cases.

**Table 2: Isomerization reaction for compounds 2.4-2.5**

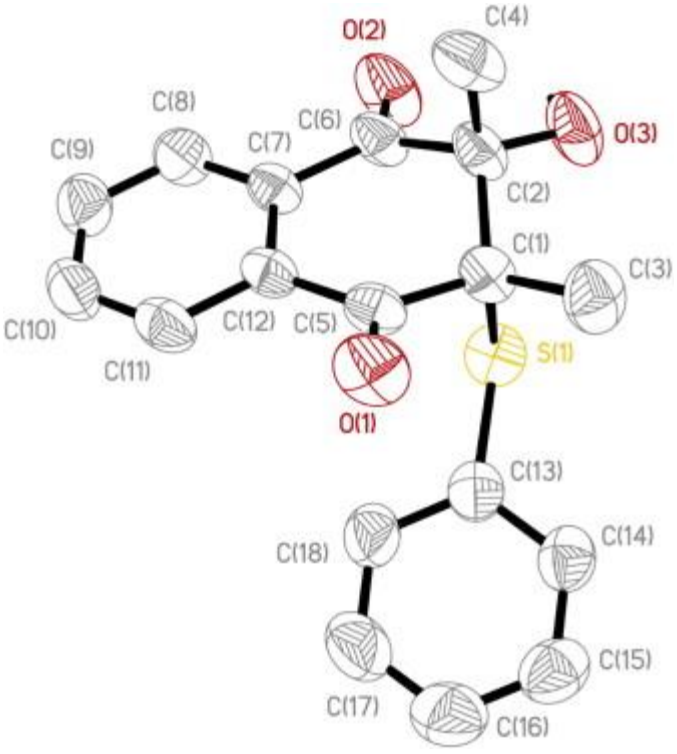
| Entry | Substrate | Base | *anti*/*syn* |
|---|---|---|---|
| 1 | *anti*-**2.4** | NaSEt | 70/30 |
| 2 | *anti*-**2.4** | NaSPh | >95/5 |
| 3 | *anti*-**2.5** | NaSEt | 58/42 |
| 4 | *anti*-**2.5** | NaSPh | 38/62 |

According to the results, the observed general tendencies can be represented in the next **scheme 7**.




**Scheme 7**: General isomerization tendency

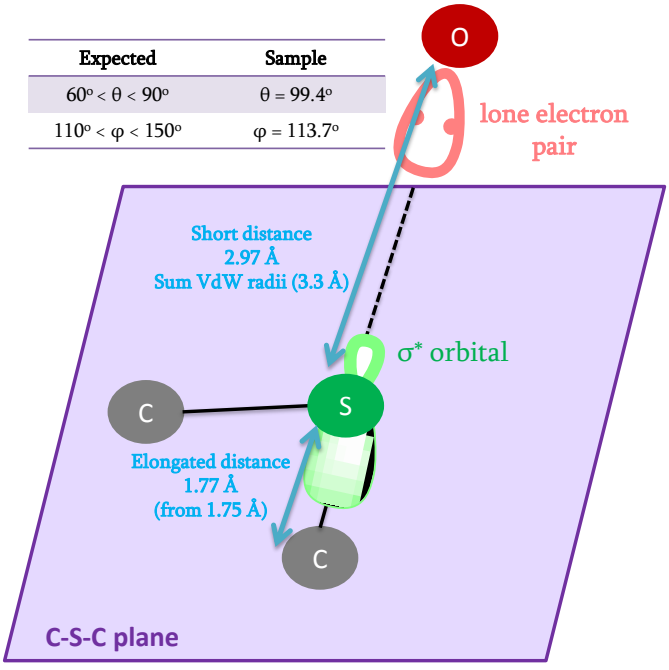
To conclude that S-O interactions are controlling the stereochemistry in sulfur compounds, (**scheme 8**).




**Scheme 8**: X-ray structure of *syn*-**2.2**

According to the angles (azimuthal $\varphi$=113.7° and polar $\theta$=99.4°) in the close contact between sulfur and oxygen of the hydroxyl, was quite similar than the case of the study of β-hydroxy-α-sulfenyl-γ-butyrolactones[5] (azimuthal φ=107° and polar θ=93°). The distance between hydroxyl oxygen and al sulfur was 2.97 Å, less than Van der Waals radii (3.3 Å). The angles and distance confirm this interaction.

The linear alignment of the C–S covalent bond and the coordinating hydroxyl oxygen should allow an effective orbital interaction between the oxygen lone electron pair and the σ* orbital of the S–C bond, which may elongate the S–C bond (1.77 Å for *syn*-**2.2**, 1.75 Å for the diphenyl disulfide).




This short distance between sulfur atoms and oxygen could be accepted as a nonbonded interaction stabilizing *syn* isomer in all cases. The phenyl group in the sulfur atom position was orientated away from hydroxyl group, maximizing the interaction. Type I S-O interactions usually approach along the extension of covalent bonds to sulfur, as this case.

Other possible S-O interaction could occur between the carbonyl oxygen and sulfur atom, stabilizing with other nonbonded S-O interaction some isomers. In this case, the atomic distance was 3.55 Å and with values of angles (azimuthal φ=94.9° and polar θ=97.9°) quite different as expected for this kind of interactions. For this reason this parameters could not be attributed to S-O interaction. This might be due to the rigidity of the structure. The planar structure of the

naphthoquinones does not permit a free movement to get the correct distances and angles to contact sulfur and oxygen atoms. This might be the reason why β-hydroxy-α-sulfenyl-γ-butyrolactones give syn isomer as a single isomer while for α-hydroxy-β-sulfenyl-α,β-dimethyl naphthoquinones some anti-isomer is also observed.

# 3. Conclusions

It has been demonstrated that a 1,4 sulfur-oxygen intramolecular interaction of divalent sulfur and hydroxyl oxygen is the driving force for the *syn* stereochemical preference of α-hydroxy-β-sulfenyl-α,β-dimethyl naphthoquinones when submitted to basic isomerization. This has been demostrated by X-rays parameters of a single crystal structure and experimental isomerization reactions. When sulfur atom was replaced by oxygen atom, then the results were completely inverted, being *anti* isomers the major ones.

This study complemented the understanding of the role played by this subtle noncovalent interaction in determining the biochemical processing of vitamin-K-epoxide during blood coagulation.

# 4. Experimental information

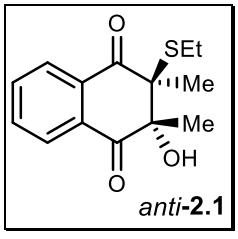
All solvents used in reactions were freshly distilled from appropriate drying agents before use. $^{1}H$ NMR spectra and $^{13}C$ NMR spectra were measured in $CDCl_3$ ($^{1}H$, 7.24 ppm; $^{13}C$ 77.0 ppm) solution at 30 °C on a 300 MHz or a 500 MHz NMR spectrometer. Mass spectra were measured in a hybrid quadrupole-t-TOF mass spectrometer operating at a resolution ca. 15000 FWHM (W-mode) with an orthogonal Z-spray-electrospray interface was used. The drying gas as well as nebulizing gas was nitrogen at a flow of 400 and 60 L/h, respectively. The temperature of the source block was set to 120 °C and the desolvation temperature to 150 °C. A capillary voltage of 3 kV was used in the positive scan mode, and the cone voltage was set to 15 V. Sample solutions were infused via syringe pump directly connected to the ESI source at a flow rate of 10 μL/min. ESI mass spectra were dominated by the presence of sodium adducts of the target compound. For the accurate mass measurements, a 2 mg/L standard solution of leucine enkephalin was introduced via the lock spray needle at a cone voltage set to 45 V and a flow rate of 30 μL/min. IR spectra were recorded as oil films or KBr discs or NaCl pellets on a FT-IR spectrometer. EM Science Silica Gel 60 was used for column

chromatography while TLC was performed with precoated plates (Kieselgel 60, F254, 0.25 mm). Unless otherwise specified, all reactions were carried out under nitrogen atmosphere with magnetic stirring.

General experimental procedure for the preparation of thioethers *anti*-**2.1** and *anti*-**2.2**

To an ice-bath cold solution of 2,3-dimethyl-1,4-napht-hoquinone-2,3-epoxide (202 mg, 1.0 mmol) in dry acetonitrile (2.0 mL) was added drop wise the corresponding thiol (3.0 mmol) and then triethylamine (140 μL, 1.0 mmol). The resulting mixture was stirred cold with an ice-bath for 3.5 h. Then was quenched with dichloromethane (15 mL) and 5% $Na_2CO_3$ (15 mL). The organic layer was separated, the aqueous layer was extracted with dichloromethane (3×15 mL), and then the organic layers were dried ($Na_2SO_4$) and concentrated. The crude was purified through chromatography (silica-gel, hexanes/ethyl acetate (7:3)) to afford the desired compound. The resulting solid mixture was recrystallized from hexanes.

*(2R,3S)-2-(Ethylthio)-3-hydroxy-2,3-dimethyl-2,3-dihydronaphthalene-1,4-dione* anti-**2.1**

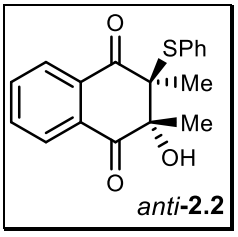


*anti*-**2.1**

Recrystallized from hexanes gave white crystals, mp 91–92 °C (lit.[2] 93–93.5 °C) (Yield=251 mg, 95%). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 7.97 (1H, d, $J$=7.3 Hz), 7.85

(1H, d, *J*=7.2 Hz), 7.57–7.64 (2H, m), 3.84 (1H, br s), 2.44 (m, 1H), 2.16 (m, 1H), 1.64 (3H, s), 1.55 (3H, s), 1.00 (3H, t, *J*=7.4 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$195.2, 192.8, 134.1, 133.8, 132.7, 132.2, 127.1, 126.8, 80.2, 60.9, 23.8, 18.5, 16.3, 13.8 ppm. IR (NaCl) $\delta$3018, 2951, 2930, 1696, 1595, 1539, 1455, 1371, 1281, 1188, 1110, 1016, 975, 937 $cm^{-1}$. HRMS *m*/*z* calcd for $C_{14}H_{16}O_3SNa$ $[M+Na^+]$: 287.0718, found: 287.0720.

*(2R,3S)-2-Hydroxy-2,3-dimethyl-3-(phenylthio)-2,3-dihydronaphthalene-1,4-dione* anti-**2.2**



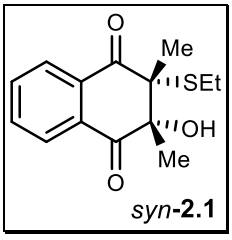

Recrystallized from hexanes gave white crystals, mp 109–112 °C (lit.[2] 115.5–116.5 °C) (Yield=287 mg, 92%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.87–7.98 (2H, m), 7.62–7.67 (2H, m), 7.13–7.32 (2H, m), 3.64 (1H, s), 1.68 (3H, s), 1.58 (3H, s). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 194.9, 192.7, 137.0, 134.2, 133.8, 133.5, 132.2, 129.9, 128.8, 127.2, 127.0, 80.2, 64.4, 18.5, 17.0 ppm. IR (NaCl) $\delta$ 3035, 2929, 1698, 1601, 1507, 1370, 1113, 1047, 949, 888 $cm^{-1}$. HRMS *m*/*z* calcd for $C_{18}H_{16}O_3SNa$ $[M+Na^+]$: 335.0718, found: 335.0719.

General experimental procedure for the preparation of thioethers *syn*-**2.1**, *syn*-**2.2** and *syn*-**2.3**

To an ice-bath cold solution of 2,3-dimethyl-1,4-naphtho-quinone-2,3-epoxide (202 mg, 1.0 mmol) in dry tetrahydrofuran (5.0 mL) was added drop in one

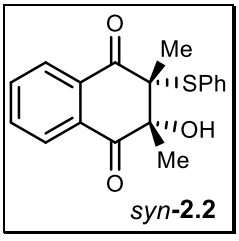
portion the corresponding sodium thiolate (1.0 mmol). The resulting mixture was stirred cold with an ice-bath for 1 h. Then it was quenched with dichloromethane (15 mL) and water (15 mL). The organic layer was separated, the aqueous layer was extracted with dichloromethane (3×15 mL), and then the organic layers were dried ($Na_2SO_4$) and concentrated. The crude was purified through chromatography (silica-gel, hexanes/ethyl acetate (7:3)) to afford the desired compound.

*(2S,3S)-2-(Ethylthio)-3-hydroxy-2,3-dimethyl-2,3-dihydronaphthalene-1,4-dione* syn-**2.1**



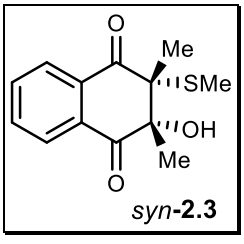

Yield=243 mg, 92%. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.03–8.06 (1H, m), 7.96–7.99 (1H, m), 7.64–7.74 (2H, m), 4.11 (1H, br s), 2.34 (m, 1H), 2.07 (m, 1H), 1.64 (3H, s), 1.26 (3H, s), 0.94 (3H, t, *J*=7.5 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 198.8, 191.5, 134.8, 134.1, 133.1, 131.1, 127.5, 126.7, 80.0, 62.5, 24.7, 23.7, 15.2, 13.7 ppm. IR (NaCl) $\delta$ 3040, 2930, 1730, 1600, 1442, 1332, 1225, 1159, 1068, 1025, 859, 777, 750, 597 cm$^{-1}$. HRMS *m*/*z* calcd for $C_{14}H_{16}O_3SNa$ [M+Na$^+$]: 287.0718, found: 287.0715.

*(2S,3S)-2-Hydroxy-2,3-dimethyl-3-(phenylthio)-2,3-dihydronaphthalene-1,4-dione* syn-**2.2**




Recrystallized from $CH_2Cl_2$–hexanes gave white crystals, mp 86–87 °C (lit.[2] 85.5–89 °C) (Yield=281 mg, 90%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.10 (1H, m), 7.92 (1H, m), 7.71–7.75 (2H, m), 7.27 (1H, t, *J*=7.4 Hz), 7.16 (2H, t, *J*=7.8 Hz), 7.06 (2H, d, *J*=7.3 Hz), 4.37 (1H, s), 1.66 (3H, s), 1.34 (3H, s). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 198.9, 191.3, 136.9, 135.0, 134.1, 134.0, 131.0, 129.9, 129.4, 128.8, 127.6, 126.9, 80.1, 66.5, 25.2, 16.0 ppm. IR (NaCl) $\delta$ 3060, 2980, 2935, 1885, 1731, 1563, 1442, 1330, 1253, 1160, 1075, 1025, 897, 859, 776, 691 $cm^{-1}$. HRMS *m*/*z* calcd for $C_{18}H_{16}O_3SNa$ [$M+Na^+$]: 335.0718, found: 335.0723.

*(2S,3S)-2-Hydroxy-2,3-dimethyl-3-(methylthio)-2,3-dihydronaphthalene-1,4-dione* syn-**2.3**



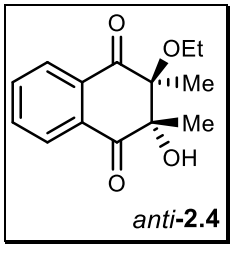

Recrystallized from $CH_2Cl_2$–hexanes gave orange solid, mp 81–83 °C (Yield=223 mg, 89%). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.01–8.04 (1H, m), 7.93–7.96 (1H, m), 7.62–7.72 (2H, m), 4.15 (1H, br s), 1.70 (3H, s), 1.57 (3H, s), 1.26 (3H,

s). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 198.9, 190.1, 134.9, 134.1, 132.9, 131.0, 127.4, 126.7, 80.0, 61.9, 25.0, 14.1, 12.4 ppm. IR (NaCl) $\delta$ 3019, 2987, 2937, 1680, 1592, 1507, 1455, 1386, 1311, 1292, 1263, 1174, 1005, 885, 713 $cm^{-1}$. HRMS *m/z* calcd for $C_{13}H_{14}O_3SNa$ [$M+Na^+$]: 273.0561, found: 273.0562.

## General experimental procedure for the preparation of ethers **2.4**–**2.5**

To an ice-bath cold solution of 2,3-dimethyl-1,4-naphtho-quinone-2,3-epoxide (506 mg, 2.5 mmol) in dry dichloromethane (12.5 mL) and methanol (12.5 mL) was added drop wise boron trifluoride etherate (0.48 mL, 3.8 mmol). The resulting mixture was heated at 50 °C for 14 days. Then was quenched with saturated aqueous sodium bicarbonate (15 mL) and extracted with dichloromethane (3×15 mL), and then the organic layers were washed (brine), dried ($Na_2SO_4$) and concentrated. The crude was purified through chromatography (silica-gel, hexanes/ethyl acetate (7:3)) to afford the desired compound.

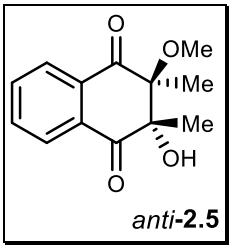
*(2S,3S)-2-Ethoxy-3-hydroxy-2,3-dimethyl-2,3-dihydronaphthalene-1,4-dione* anti-**2.4**




White needles, mp 67–70 °C (Yield=372 mg, 60%) (Quantitative yield based on recovered starting material).$^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 7.90–7.93 (2H, m), 7.59–

7.64 (2H, m), 4.01 (1H, br s), 3.47–3.53 (1H, m), 3.22–3.28 (1H, m), 1.38 (3H, s), 1.34 (3H, s), 0.90 (3H, t, *J*=7.1 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 197.35, 197.14, 134.3, 134.0, 133.3, 132.5, 126.9, 126.8, 85.0, 81.0, 60.1, 19.3, 15.5, 13.9 ppm. IR (NaCl) $\delta$ 3046, 2981, 1697, 1507, 1456, 1276, 1054, 984, 707, 667 $cm^{-1}$. HRMS *m*/*z* calcd for $C_{14}H_{16}O_4Na$ [M+$Na^+$]: 271.0946, found: 271.0948.

*(2S,3S)-2-Hydroxy-3-methoxy-2,3-dimethyl-2,3-dihydronaphthalene-1,4-dione* anti-**2.5**




White needles, mp 97–99 °C (Yield=322 mg, 55%) (Quantitative yield based on recovered starting material).$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.94–7.99 (2H, m), 7.63–7.69 (2H, m), 3.92 (1H, br s), 3.27 (3H, s), 1.37 (3H, s), 1.34 (3H, s). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 197.8, 196.8, 134.5, 134.1, 133.3, 132.2, 127.1, 127.0, 85.1, 81.3, 52.5, 20.2, 13.9 ppm. IR (NaCl) $\delta$ 3019, 2958, 2938, 1698, 1596, 1539, 1455, 1372, 1281, 1189, 1122, 1017, 938 $cm^{-1}$. HRMS *m*/*z* calcd for $C_{13}H_{14}O_4Na$ [M+$Na^+$]: 257.0790, found: 257.0792.

## Crystallographic data

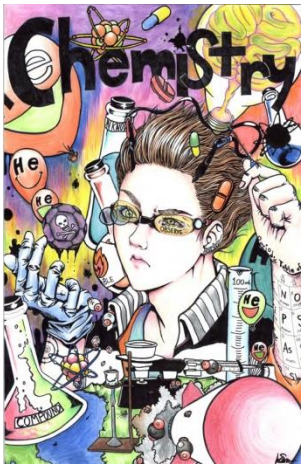
Single crystals of $C_{18}H_{16}O_3S$ were mounted on a MicroMount® polymer tip (MiteGen) in a random orientation. Data collection was performed on a SuperNova dual source equipped with a CCD Atlas detector diffractometer (Agilent Technologies). The crystal was kept at 293(2) K during data collection. Using Olex2,[47] the structure was solved with the ShelXS[48] structure solution program using Direct Methods and refined with the ShelXL[49] refinement package using Least Squares minimisation.

*Syn*-**2.2**: $C_{18}H_{16}O_3S$, M =312.37, triclinic, a = 7.7667(5) Å, b = 10.1556(6) Å, c = 10.4484(6) Å, α = 77.2900(10)°, β = 71.4970(10)°, γ = 80.5300(10)°, V = 758.37(8) Å3, T = 298(2), space group P-1 (no. 2), Z = 2, μ(MoKα) = 0.223, 8857 reflections measured, 3482 unique (Rint = 0.0164) which were used in all calculations. The final wR2 was 0.1110 (all data) and R1 was 0.0374 (>2sigma(I)).

[47] O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann, OLEX2: a complete structure solution, refinement and analysis program. J. Appl. Cryst. (2009). 42, 339-341.
[48] SHELXS-97 (Sheldrick, 1990).
[49] SHELXL, G.M. Sheldrick, Acta Cryst. 2008. A64, 112-122.

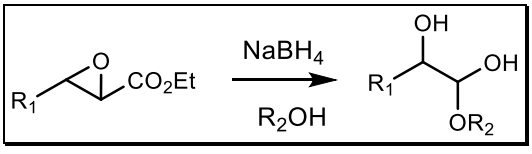
# Chapter 3:

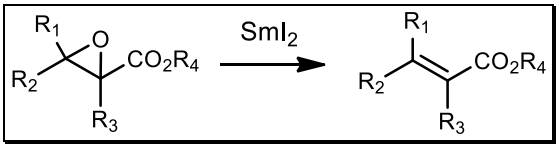
# Study of the stereoselectivity of the nucleophilic epoxidation of 3-hydroxy-2-methylene esters



# 1. Introduction

The α,β-epoxy esters are among the most interesting compounds due to the numerous transformations that can be performed with them. The opening of the oxirane ring can generate a large number of products which are interesting intermediates.[50,51,52,53,54,55,56,57,58]




[50] Chong, J. M.; Sharpless, K. B. *Tetrahedron Lett.* **1985**, 26, 4683-4686
[51] Meth-Cohn, O.; Moore, C.; Taljaard, H. C. *J. Chem. Soc., Perkin Trans.* 1 **1988**, 2663-2674
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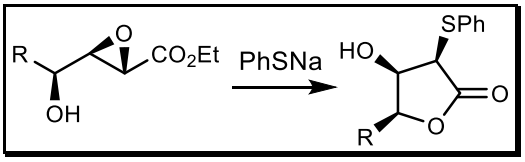
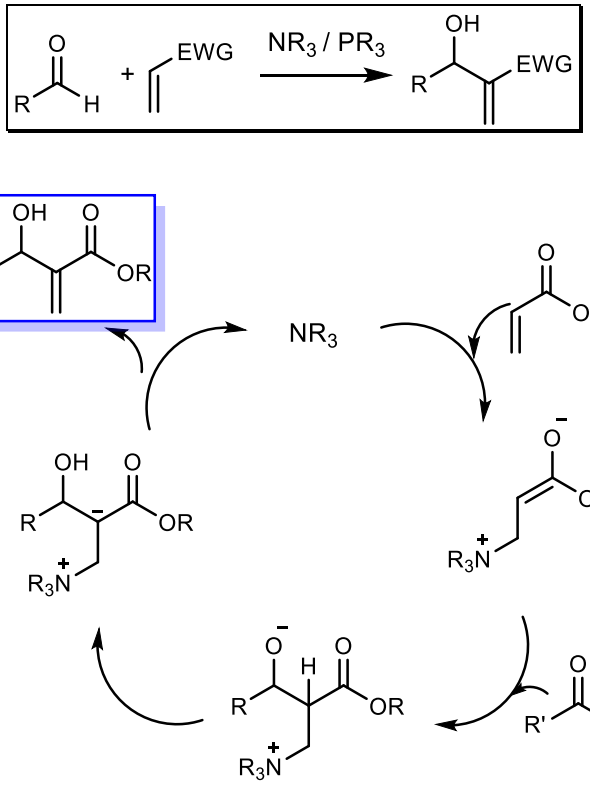
[53] Lanier, M.; Pastor, R. *Tetrahedron Lett.* **1995**, 36, 2491-2492
[54] Righi, G.; Rumboldt, G.; Bonini, C. *J. Org. Chem.* **1996**, 61, 3557-3560
[55] Concellón, J. M.; Bardales, E. *Org. Lett.* **2002**, 4, 189-191.
[56] Concellón, J. M.; Bardales, E.; Llavona, R. *J. Org. Chem.* **2003**, 68, 1585-1588
[57] Rodríguez, S.; Izquierdo, F.; Lopez, I.; Gonz alez, F. V. *Tetrahedron* **2006**, 62, 11112-11123
[58] Lopez, I.; Izquierdo, J.; Rodríguez, S.; González, F. V. *J. Org. Chem.* **2007**, 72, 6614-6617




**Scheme 1**. General α,β-epoxy esters applications

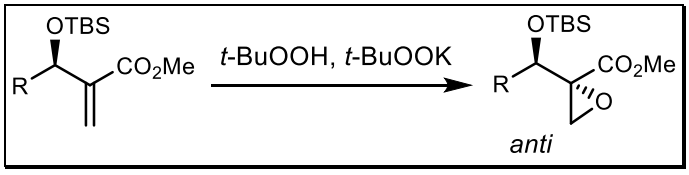
Morita-Baylis-Hillman (MBH) is an important organic reaction forming a C-C bond between and activated alkene and a carbonylic compound. The reaction is performed in the presence of a tertiary amine or a phosphine. The reaction and mechanism is summarized in **scheme 2**. It has also been used in the total synthesis of interesting natural products.[59,60]




**Scheme 2**. General reaction and mechanism of MBH reaction

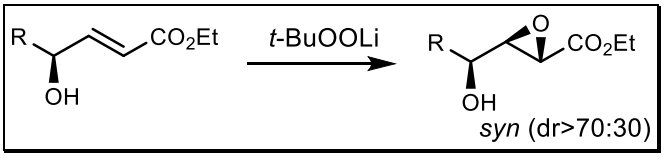
[59] Bailey, M.; Marko, I. E.; Ollis, W. D.; Rasmussen, P. R. *Tetrahedron Lett.* **1990**, 31, 4509-4512
[60] Bailey, M.; Staton, I.; Ashton, P. R.; Mark_o, I. E.; Ollis, W. D. *Tetrahedron: Asymmetry* **1991**, 2, 495-509

Some epoxidation reactions of MBH adducts have been previously reported. It is remarkable the importance of the hydroxyl group in this system due to the drastically effect on the diastereoselectivity depending on if it is protected or not. A. Meyers reported,[61] as illustrated in **scheme 3** the preparation of *anti* diastereoisomer epoxide in high selectivity when the hydroxyl group is silylated.




**Scheme 3**. Epoxidation of O-silylated MBH adduct

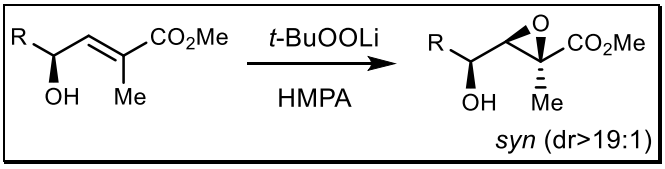
We previously reported, as it is shown in **scheme 4**, the diastereoselective nucleophilic epoxidation of γ-hydroxy-α,β-unsaturated esters using lithium *tert*-butoxide as an oxidant. In this case *syn* isomer was obtained (dr> 70:30).




**Scheme 4**. Epoxidation of γ-hydroxy-α,β-unsaturated esters

We also observed that the reaction is more stereoselective if there is a substituent at the α-position, also again in favor if *syn* isomer (dr > 19:1). It can be observed in **scheme 5**.

[61] Svenda, M.; Myers, A. G. *Org. Lett.* **2009**, 11, 2437-2440

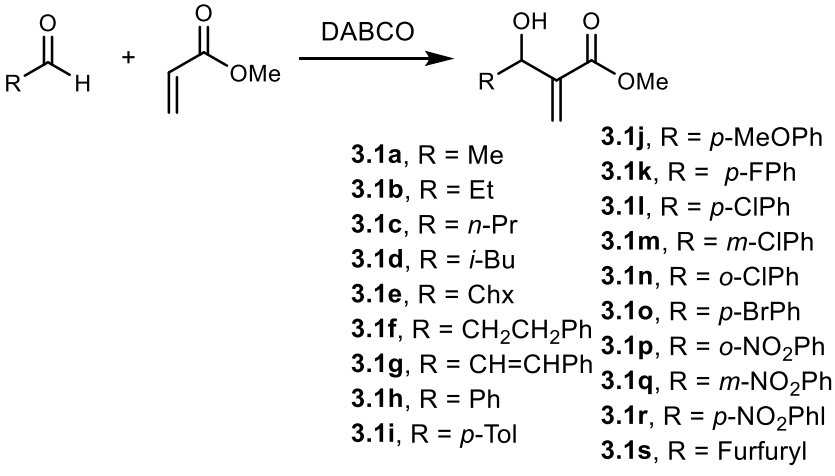



**Scheme 5**. Effect double bound substitution

Due to the interesting scope of possibilities of the α,β-epoxy esters, the transformations of MBH adducts to prepare natural products and the lack of a deep study of the diastereoselectivity of the epoxidation reaction of MBH adducts, we decided to explore the stereoselectivity of the epoxidation of 3-hydroxy-2-methylene esters.

# 2. Results and discussion

The stereoselectivity of the epoxidation of 3-hydroxy-methylene carboxylate esters was studied. A large range of alkyl and aryl aldehydes was selected as representative electrophiles.




**Scheme 6**: Preparation of MBH adducts

For the preparation of the substrates, a whole study of different MBH reaction conditions was performed, due to the large number of conditions

described on literature and noting that the reaction conditions depends on the substrate. The results are summarized in **table 1**.

**Table 1. MBH reaction results**

| Entry | Substrate | Conditions[a] | Yield (%) |
|---|---|---|---|
| 1 | **3.1a** | dioxane/ $H_2O$ (1:1), 10 M, 48 h, r.t. | 99 |
| 2 | **3.1b** | dioxane/ $H_2O$ (1:1), 10 M, 48 h, r.t. | 70 |
| 3 | **3.1c** | dioxane/ $H_2O$ (1:1), 10 M, 48 h, r.t. | 99 |
| 4 | **3.1d** | dioxane/ $H_2O$ (1:1), 10 M, 48 h, r.t. | 85 |
| 5 | **3.1e** | dioxane/ $H_2O$ (1:1), 10 M, 48 h, r.t. | 81 |
| 6 | **3.1f** | dioxane/ $H_2O$ (1:1), 10 M, 48 h, r.t. | 99 |
| 7 | **3.1g** | dioxane/ $H_2O$ (1:1), 10 M, 48 h, r.t. | 99 |
| 8 | **3.1h** | dioxane/ $H_2O$ (1:1), 12 M, 48 h, r.t. | 99 |
| 9 | **3.1i** | solvent-free, 4 days, r.t. | 82 |
| 10 | **3.1j** | solvent-free, 5 weeks, r.t. | 77 |
| 11 | **3.1k** | dioxane/ $H_2O$ (1:1), 10 M, 36 h, r.t. | 99 |
| 12 | **3.1l** | solvent-free, 5 days, r.t. | 94 |
| 13 | **3.1m** | DMSO 7 M, 4 days, r.t. | 99 |
| 14 | **3.1n** | DMSO 7 M, 4 days, r.t. | 99 |
| 15 | **3.1o** | dioxane/ $H_2O$ (1:1), 10 M, 36 h, r.t. | 89 |
| 16 | **3.1p** | dioxane/ $H_2O$ (1:1), 10 M, 16 h, r.t. | 95 |
| 17 | **3.1q** | dioxane/ $H_2O$ (1:1), 10 M, 16 h, r.t. | 87 |
| 18 | **3.1r** | dioxane/ $H_2O$ (1:1), 10 M, 3 h, r.t. | 83 |
| 19 | **3.1s** | dioxane/ $H_2O$ (1:1), 10 M, 20 h, r.t. | 85 |

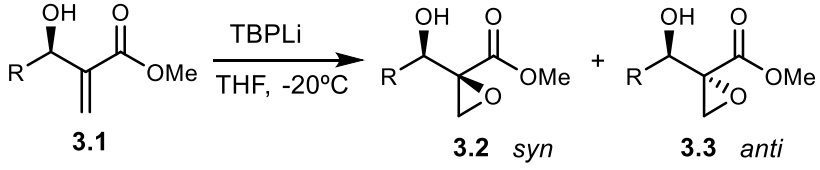
[a] Using DABCO as base

Aldehydes were treated with acrylates using 1,4-Diazabicyclo[2.2.2]octane (DABCO) in all cases and the reactions were performed at room temperature. In

most cases, a 1/1 mixture of dioxane/water was used as solvent as previously described.[62] The reaction worked better if carried out at 10M concentration (respect to aldehyde), this is a higher concentration than the one reported.[62] Generally speaking, chemical yields were very good to excellent in all cases. The reaction times were between 3 hours to 5 weeks, depending on the substrate.

Compounds **3.1m** and **3.1n** were prepared with dimethylsulfoxide as a solvent due to best results are obtained. It was remarkable that compounds **3.1i** and **3.1j** were prepared under free solvent conditions and for longer time to get higher chemical yield.

The epoxidation of MBH compounds **3.1a-s** was carried out using lithium *tert*-butylperoxide (*in situ* prepared by adding 2 equivalents of *tert*-butyl hydrogen peroxide and 1.7 equivalents of methyl lithium) in tetrahydrofuran at -20 $^{0}$C affording the desired epoxide in moderate to good yields.[2,8,9] The nucleophilic epoxidation of MBH adducts generated a mixture of *syn* /*anti* epoxides in all cases, as described in **scheme 7**. The main results of the epoxidation reactions are illustrated in **table 2**.




**Scheme 7**: Preparation of MBH adducts

[62] Yu, C.; Liu, B.; Hu, L. *J. Org. Chem.* **2001**, 66, 5413-5418

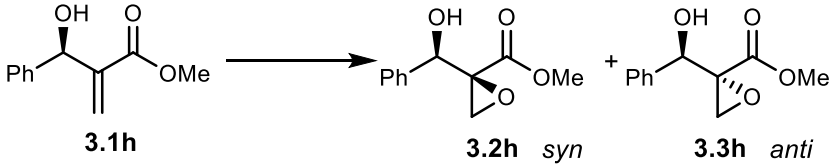
Table 2: Epoxidation reaction

| Entry | R | 3.2 : 3.3 ratio | Yield (%) |
|---|---|---|---|
| 1 | Me | 67 : 33 | 72 |
| 2 | Et | 76 : 24 | 70 |
| 3 | *n*-Pr | 81 : 19 | 79 |
| 4 | *i*-Bu | 81 : 19 | 71 |
| 5 | Chx | 92 : 8 | 85 |
| 6 | $PhCH_2CH_2$ | 77 : 23 | 59 |
| 7 | PhCH=CH | 53 : 47 | 47 |
| 8 | Ph | 93 : 7 | 68 |
| 9 | *p*-Tol | 89 : 11 | 82 |
| 10 | *p*-MeOPh | 92 : 8 | 73 |
| 11 | *p*-FPh | 90 : 10 | 65 |
| 12 | *p*-ClPh | 84 : 16 | 52 |
| 13 | *m*-ClPh | 92 : 8 | 38 |
| 14 | *o*-ClPh | 92 : 8 | 52 |
| 15 | *p*-BrPh | 90 : 10 | 68 |
| 16 | *o*-$NO_2$Ph | 83 : 17 | 43 |
| 17 | *m*-$NO_2$Ph | 80 : 20 | 60 |
| 18 | *p*-$NO_2$Ph | 91 : 9 | 65 |
| 19 | Furfuryl | 93 : 7 | 69 |

Results were analyzed according to the R substitution. In all cases *syn* isomer was the major product for the epoxidation. Aliphatic products, **entries 1-6**, were obtained in good chemical yields (59% to 85%). For this series, the higher steric volume of R substituent the higher stereoselectivity is observed (67:33 to 92:8). The alkenyl group, **entry 7**, is obtained in low chemical yield (47%) and poor diastereoselectivity (53:47). In the aromatic series, in general, *syn* isomer was

obtained in good chemical yields (38% to 82%) and very good selectivity (80:20 to 93:7).

Next other oxidants were explored in order to increase the chemical yield and selectivity. Compound **3.1h** was chosen as the substrate to assay new epoxidation conditions. New results can be seen in **table 3**.




**Scheme 8**: Epoxidation reaction of compound **3.1h**

**Table 3: Epoxidation of 3.1h using other oxidants**

| Entry | Conditions | 3.2h : 3.3h ratio | Yield (%) |
|---|---|---|---|
| 1[a] | TBPLi | 88:12 | 66 |
| 2 | CMPLi | 91:9 | 72 |
| 3 | *m*-CPBA | 90:10 | 28 |
| 4 | *m*-CPBA | 88:12 | 80 |
| 5 | *m*-CPBA | — | NR |
| 6[b] | TBPNa | 85:15 | 41 |
| 7[c] | TBPNa | 87:13 | 62 |
| 8 | TBPK | 83:17 | 61 |

[a] 0.8 equiv of MeLi + 1.5 equiv of TBHP
[b] NaH + TBHP
[c] NaO*t*Bu + TBHP

For **entry 1**, lithium *tert*-butyl peroxide was used but decreasing the amount of base (0.8 equivalents of methyl lithium and 1.5 equivalents of *tert*-butyl hydrogen peroxide in tetrahydrofuran at -20 °C for 20 h) but lower selectivity

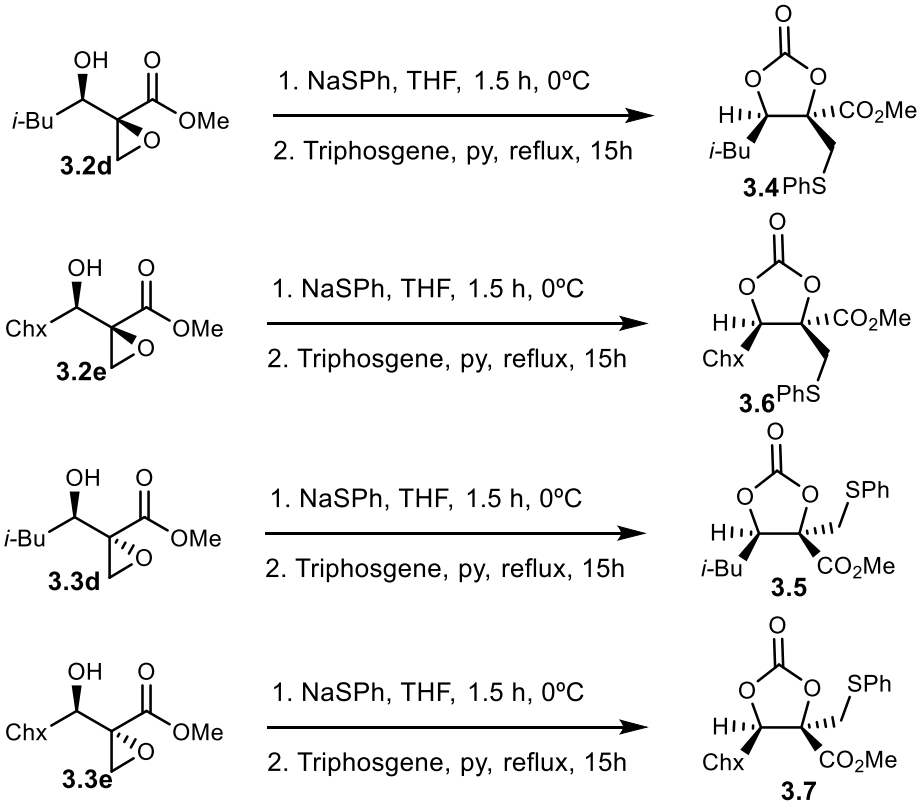
(88:12) and chemical yield (66%) were observed. When lithium cumyl peroxide (1.5 eq cumyl hydrogen peroxide and 1.1 equivalents of methyl lithium) was used (**entry 2**), in the same condition as before, similar selectivity (91:9) and a sligh increase of chemical yield (72%) were observed.

When sodium *tert*-butyl peroxide was used (readily prepared from 2 equivalens of *tert*-butyl hydrogen peroxide and 1 equivalent of sodium *tert*-butoxide) as an oxidant (**entry 6**) in tetrahydrofuran 0 ºC for 3 hours, poor chemical yield (41%) and lower selectivity (85:15) were observed. Same conditions than **entry 6** but a less amount of base were also tested as depicted in **entry 7**, (2 equivalents of *tert*-butyl hydrogen peroxide and 0.25 equivalents of sodium *tert*-butoxide) which increased the chemical yield (62%) and selectivity (87:13) respect to **entry 6**. When potassium *tert*-butyl peroxide (2 equivalents) was used (**entry 8**) in tetrahydrofuran at 0 ºC for 3 hours, poor chemical yield (61%) and lower selectivity (83:17) were observed compared with lithium and sodium series.

Finally an epoxidation using an electrophilic oxidant was also evaluated: 2.1 equivalents of *meta*-chloroperbenzoic acid were used in dichloromethane for 96 hours at room temperature (**entry 3**) and good selectivity (90:10) and poor chemical yield (28%) were oberved. These conditions but increasing temperature to 70 ºC in a sealed tube (**entry 4**) led to the same selectivity (88:12) and an improvement of chemical yield (80%). When 2.5 equivalents of *meta*-chloroperbenzoic acid were employed in the presence of 1.3 equivalents of potassium carbonate for 96 hours at room temperature (**entry 5**),[63] starting material was recovered. It was remarkable that all chemical yields were calculated after chromatographic purification of the crude.

[63] García-Ruano, J. L.; Fajardo, C.; Fraile, A.; Martí, M. R. *J. Org. Chem.* **2005**, 70, 4300-4306

To confirm the stereochemistry, compounds **3.2b** and **3.2h** were compared with previous reported data,[63,64] and epoxides **3.2d**, **3.3d**, **3.2e** and **3.3e** were derivatized into cyclic carbonates.

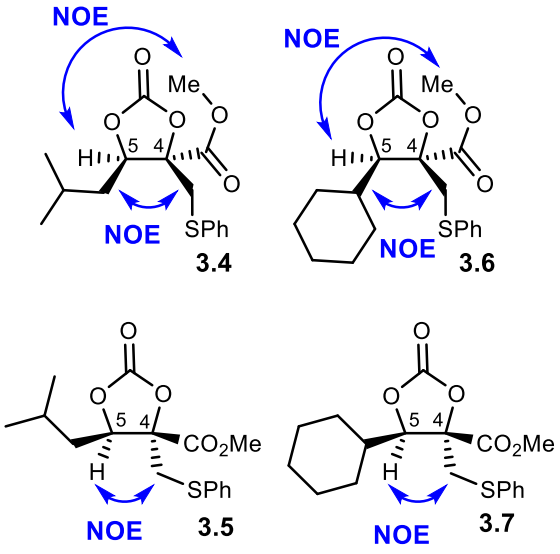



**Scheme 9.** Preparation of cyclic compounds

As it is illustrated in **scheme 9**, epoxides **3.2d**, **3.3d**, **3.2e** and **3.3e** were treated with sodium thiophenolate readily prepared by previously mixing 1.5 equivalents of sodium hydride with 2 equivalents of thiophenol for 15 minutes cold with an ice-bath. Then the epoxide was added and reaction was carried out at 0 ºC for 15 min in THF at room temperature for 1,5 hours. The resulting diol was cyclized as carbonate by adding triphosgene and pyridine.

[64] Adam, W.; Braun, M.; Griesbek, A.; Lucchini, V.; Staab, E.; Will, B. *J. Am. Chem. Soc.* **1989**, 111, 203-212

The cyclic carbonates **3.4-3.7** were isolated in good chemical yields (70% - 80%) and NOE experiments were carried out to make stereochemical assignments as illustrated in **scheme 10**. Both *syn* isomers **3.4** and **3.6** (from **3.2d** and **3.2e**, respectively) had a strong NOE effect between H-5 and the methyl ester group and other NOE effect between methylene bound to sulfur and H-alkyl chain in 5-position. On the other hand, *anti* isomers **3.5** and **3.7** (from **3.3d** and **3.3e,** respectively**)** had a strong NOE effect between H-5 and the methylene bound to sulfur.




**Scheme 10**: NOE experiments on carbonates **3.4-3.7**

# 3. Conclusions

A study of the diastereoselectivity of the epoxidation of 3-hydroxy-2-methylene esters has been performed. An extensive experimental work was done for the optimization of MBH reaction as applied to a range of alkyl and aryl aldehydes. Most of MBH adducts were compared with previously published data.[65,66,67,68,69] The study of epoxidation conditions of MBH adducts was reached. The diastereoselectivity preference of this nucleophilic epoxidation to *syn* isomer is confirmed in all of cases, complementing the other literature studies. Finally a ring opening transformation with thiophenol and triphosgene led to the formation of cyclic carbonates compounds for stereochemical assignments.

[65] Lee, K.; Loh, T. *Chem. Commun.* **2006**, 4209-4211
[66] Brzezinski, L. J.; Rafel, S.; Leahy, J. W. *Tetrahedron* **1997**, 53, 16423-16434
[67] Mi, X.; Luo, S.; Cheng, J. *J. Org. Chem.* **2005**, 70, 2338-2341
[68] Aggarwal, V. A.; Emme, I.; Fulford, S. Y. *J. Org. Chem.* **2003**, 68, 692-700
[69] Guo, Y.; Shao, G.; Li, L.;Wu,W.; Li, R.; Li, J.; Song, J.; Qiu, L.; Prashad, M.; Kwong, F. Y. *Adv. Synth. Catal.* **2010**, 352, 1539-1553

# 4. Experimental information

## General experimental methods

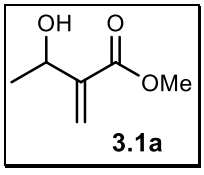
All solvents used in reactions were freshly distilled from appropriate drying agents before use. $^{1}H$ NMR spectra and $^{13}C$ NMR spectra were measured in $CDCl_3$ ($^{1}H$, 7.24 ppm; $^{13}C$ 77.0 ppm) solution at 30 °C on a 300 MHz or a 500 MHz NMR spectrometer. IR spectra were recorded as oil films or KBr discs or NaCl pellets on a FT-IR spectrometer. EM Science Silica Gel 60 was used for column chromatography while TLC was performed with precoated plates (Kieselgel 60, F254, 0.25 mm). Unless otherwise specified, all reactions were carried out under argon atmosphere with magnetic stirring.

## General experimental procedure for the preparation of compounds **3.1a–s**

To a solution of aldehyde (1 mmol) in dioxane/water (1:1) (0.1 mL) was added methyl acrylate (3 mmol) and DABCO (1 mmol). The reaction was

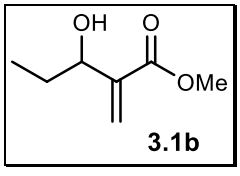
monitored by TLC. Upon completion, water (70 mL) was added and poured onto a separatory funnel and extracted with ethyl ether or dichloromethane (3×30 mL), dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure. The crude was purified through chromatography (silica-gel, hexanes/ethyl acetate (8:2), (6:4)) to afford the desired compound.

*Methyl 3-hydroxy-2-methylenebutanoate* ***3.1a***[16]



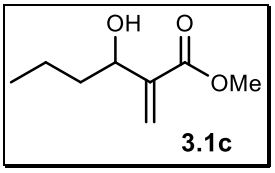

$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 6.19 (1H, s), 5.81 (1H, s), 4.59 (1H, q, $J$=6.5 Hz), 3.76 (3H, s), 2.61 (1H, br s), 1.36 (3H, d, $J$=6.5 Hz).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 167.1, 143.6, 124.0, 67.2, 51.8, 22.1 ppm.

*Methyl 3-hydroxy-2-methylenepentanoate* ***3.1b***[17]



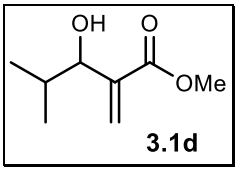

$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 6.22 (1H, s), 5.78 (s, 1H), 4.31 (1H, t, $J$=7.0 Hz), 3.76 (3H, s), 2.43 (1H, br s), 1.73–1.61 (2H, m), 0.93 (3H, t, $J$=7.4 Hz).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 167.0, 142.3, 124.7, 72.0, 51.5, 29.0, 10.0 ppm.

*Methyl 3-hydroxy-2-methylenehexanoate* ***3.1c***[18]



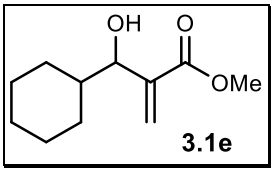

$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 6.20 (1H, s), 5.78 (1H, s), 4.38 (1H, t, *J*=6.5 Hz), 3.76 (3H, s), 2.41 (1H, br s), 1.63–1.58 (2H, m), 1.49–1.45 (1H, m), 1.31–1.38 (1H, m), 0.90 (3H, t, *J*=6.7 Hz).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 167.0, 142.5, 124.7, 71.3, 52.0, 38.5, 19.0, 14.0 ppm.

*Methyl 3-hydroxy-5-methyl-2-methylenehexanoate* ***3.1d***[18]




$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 6.18 (1H, s), 5.78 (1H, s), 4.45 (1H, dd, *J*=8.5, 4.3 Hz), 3.76 (3H, s), 2.40 (1H, br s), 1.80–1.75 (1H, m), 1.58–1.51 (1H, m), 1.44–1.38 (1H, m), 0.92 (6H, m).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$167.0, 142.8, 124.6, 71.3, 69.9, 51.9, 45.5, 24.8, 23.3, 21.8 ppm.

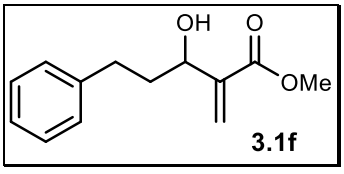
*Methyl 2-(cyclohexyl(hydroxy)methyl)acrylate* ***3.1e***[19]




$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 6.23 (1H, s), 5.71 (1H, s), 4.06 (1H, d, *J*=7.2 Hz), 3.76 (3H, s), 2.44 (1H, br s), 1.94 (1H, m), 1.50–1.76 (5H, m), 1.24–0.92 (5H, m).$^{13}$C

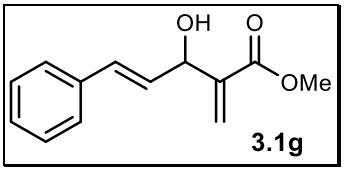
NMR (125 MHz, $CDCl_3$) $\delta$ 167.0, 141.2, 126.0, 53.4, 52.0, 42.4, 29.8, 28.1, 26.3, 26.1, 25.9 ppm.

*Methyl 3-hydroxy-2-methylene-5-phenylpentanoate* ***3.1f***[20]



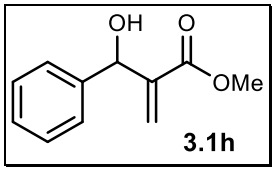

$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.30–7.17 (5H, m), 6.24 (1H, s), 5.81 (1H, s), 4.42 (1H, dd, *J*=7.5, 5.7 Hz), 3.77 (3H, s), 2.85–2.79 (1H, m), 2.73–2.69 (1H, m), 2.42 (1H, br s), 2.00–1.95 (1H, m).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 167.0, 141.8, 128.5, 125.9, 125.0, 70.1, 51.8, 38.0, 32.0 ppm.

*(**E**)-Methyl 3-hydroxy-2-methylene-5-phenylpent-4-enoate* ***3.1g***[19]



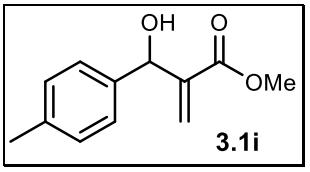

$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.39–7.22 (5H, m), 6.67 (1H, d, *J*=16.0 Hz), 6.29 (2H, m), 5.91 (1H, s), 5.13 (1H, m), 3.78 (3H, s), 2.97 (1H, br s).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 166.7, 141.3, 136.5, 131.5, 129.2, 128.5, 127.8, 126.6, 125.8, 72.1, 52.0 ppm.

*Methyl 2-(hydroxy(phenyl)methyl)acrylate* ***3.1h***[18]



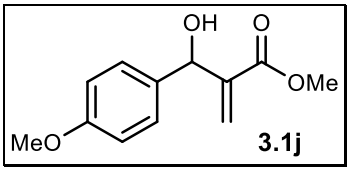
$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.38–7.26 (5H, m), 6.33 (1H, s), 5.83 (1H, s), 5.56 (1H, s), 3.72 (3H, s).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 166.7, 142.3, 141.6, 128.3, 127.7, 126.8, 125.6, 72.7, 51.8 ppm.

*Methyl 2-(hydroxy(p-tolyl)methyl)acrylate* ***3.1i***[18]



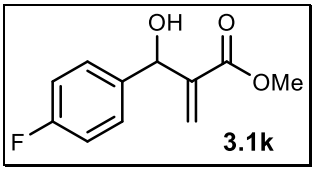

$^{1}$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.26 (2H, d, $J$=8.0 Hz), 7.15 (2H, d, $J$=8.0 Hz), 6.32 (1H, s), 5.85 (1H, s), 5.53 (1H, s), 3.71 (3H, s), 2.34 (3H, s).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 166.8, 142.1, 138.4, 137.5, 129.1, 126.5, 125.8, 73.1, 51.9, 21.1 ppm.

*Methyl 2-(hydroxy(4-methoxyphenyl)methyl)acrylate* ***3.1j***[20]



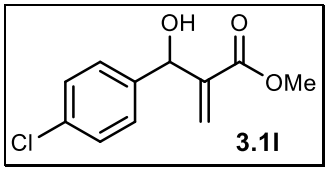

$^{1}$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.28 (2H, d, $J$=8.8 Hz), 6.86 (2H, d, $J$=8.7 Hz), 6.31 (1H, s), 5.84 (1H, s), 5.52 (1H, s), 3.79 (3H, s), 3.71 (3H, s).$^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 166.8, 159.2, 142.2, 133.5, 127.9, 125.5, 113.8, 72.7, 55.2, 51.9 ppm.

*Methyl 2-((4-fluorophenyl)(hydroxy)methyl)acrylate* ***3.1k***[20]



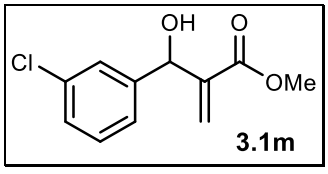
$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.33 (2H, dd, $J$=8.5, 5.5 Hz), 7.01 (2H, t, $J$=8.7 Hz), 6.32 (1H, s), 5.82 (1H, s), 5.53 (1H, s), 3.73 (3H, s), 3.02 (1H, br s). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 166.6, 162.3 (d, $J$=245 Hz), 141.9, 137.0, 128.3 (dd, $J$=7.2, 21.3 Hz), 126.0 (dd, $J$=15.0, 21.3 Hz), 115.2 (dd, $J$=12.5, 22.5 Hz), 72.6, 52.2 ppm.

*Methyl 2-((4-chlorophenyl)(hydroxy)methyl)acrylate* ***3.1l***[18]



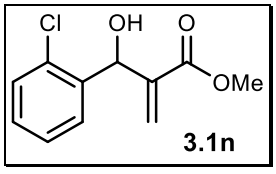

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.54 (1H, m), 7.34 (1H, m), 7.21–7.30 (2H, m), 6.32 (1H, s), 5.97 (1H, s), 5.58 (1H, m), 3.76 (3H, s), 3.26 (1H, br s).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 166.6, 141.6, 139.8, 133.6, 128.6, 127.9, 126.3, 72.7, 52.0 ppm.

*Methyl 2-((3-chlorophenyl)(hydroxy)methyl)acrylate* ***3.1m***[20]



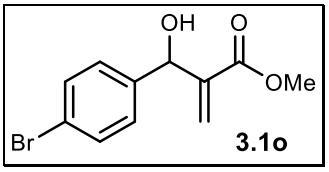

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.37 (1H, s), 7.26 (3H, m), 6.34 (1H, s), 5.83 (s, 1H), 5.51 (1H, s), 3.72 (s, 3H), 3.03 (1H, br s).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 166.5, 143.4, 141.4, 134.4, 129.7, 127.9, 126.7, 126.6, 124.7, 72.7, 52.0 ppm.

*Methyl 2-((2-chlorophenyl)(hydroxy)methyl)acrylate* ***3.1n***[20]



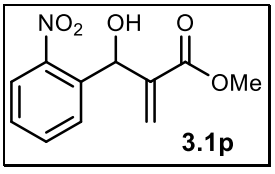
$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.54 (1H, m), 7.34 (1H, m), 7.21–7.30 (2H, m), 6.32 (1H, s), 5.97 (1H, s), 5.58 (1H, m), 3.76 (3H, s), 3.25 (1H, br s).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 166.9, 140.9, 134.5, 132.8, 128.9, 128.1, 127.0, 126.8, 68.9, 52.0 ppm.

*Methyl 2-((4-bromophenyl)(hydroxy)methyl)acrylate* ***3.1o***[20]



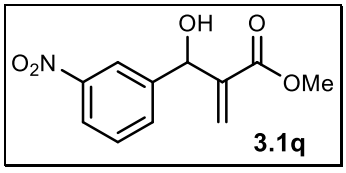

$^{1}$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.47 (2H, m), 7.25 (2H, m), 6.33 (1H, s), 5.82 (1H, s), 5.51 (1H, m), 3.73 (3H, s), 3.04 (1H, br s).$^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 166.4, 141.9, 140.6, 131.4, 128.6, 125.9, 121.6, 71.9, 51.9 ppm.

*Methyl 2-((2-nitrophenyl)(hydroxy)methyl)acrylate* ***3.1p***[18]



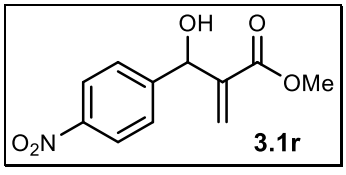

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.95 (1H, dd, $J$=8.2, 1.3 Hz), 7.75 (1H, dd, $J$=7.9, 1.3 Hz), 7.64 (1H, td, $J$=7.7, 1.3 Hz), 7.46 (1H, td, $J$=8.5, 1.4 Hz), 6.37 (1H, s), 6.20 (1H, s), 5.73 (1H, s), 3.73 (3H, s), 3.35 (1H, br s). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 163.8, 145.6, 138.5, 133.7, 130.8, 126.3, 126.0, 123.7, 121.9, 64.7, 49.5 ppm.

*Methyl 2-((3-nitrophenyl)(hydroxy)methyl)acrylate* ***3.1q***[18]



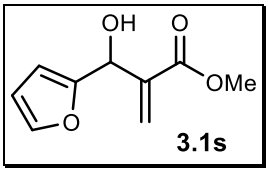

$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.26 (1H, m), 8.14 (1H, ddd, *J*=8.2, 2.3, 1.2 Hz), 7.75 (1H, m), 7.52 (1H, t,*J*=7.92 Hz), 6.41 (1H, s), 5.89 (1H, s), 5.63 (1H, s), 3.75 (3H, s), 3.25 (1H, br s).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 166.2, 148.3, 143.7, 141.0, 132.8, 129.5, 126.9, 122.8, 121.7, 72.4, 52.3 ppm.

*Methyl 2-((4-nitrophenyl)(hydroxy)methyl)acrylate* ***3.1r***[18]




$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.18 (2H, d, *J*=10.9 Hz), 7.56 (2H, d, *J*=10.9 Hz), 6.38 (1H, s), 5.86 (1H, s), 5.62 (1H, m), 3.73 (3H, s), 3.32 (1H, br s).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 166.4, 148.6, 143.7, 141.0, 127.3, 127.2, 123.6, 72.7, 52.2 ppm.

*Methyl 2-(furan-2-yl(hydroxy)methyl)acrylate* ***3.1s***[18]

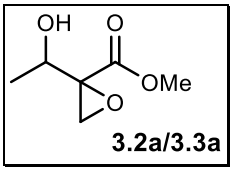



$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.35 (1H, s), 6.37 (1H, s), 6.31 (1H, m), 6.24 (1H, m), 5.93 (1H, s), 5.57 (1H, s), 3.74 (3H, s), 3.21 (1H, br s).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 166.4, 154.1, 142.3, 143.7, 139.5, 126.7, 110.4, 107.1, 67.2, 52.0 ppm.

General experimental procedure for the epoxidation of esters (**3.2a–s**)

To a –78 °C cold THF (3.5 mL) was added TBHP (3.3 M in toluene) (2 mmol) and then methyllithium (1.6 M in hexanes) (1.7 mmol). The resulting mixture was stirred at –78 °C for 15 min and then a solution of compound **3.1** (1 mmol) in THF (2 mL) was added drop wise and then the mixture was left at –20 °C (fridge) for 20 h. Then solid $Na_2SO_3$ (120 mg) was added in one portion and stirred for 15 min, then diluted with water and extracted with $Et_2O$ (3×30 mL), the organic layers were washed (brine), dried ($Na_2SO_4$), and concentrated. The crude oil was purified through chromatography (silica-gel, hexanes/EtOAc (7:3) and (1:1)).

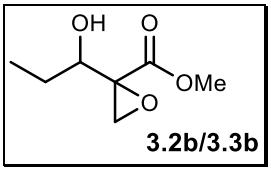
*Methyl 2-(1-hydroxyethyl)oxirane-2-carboxylate* ***3.2a/3.3a***



**3.2a/3.3a**

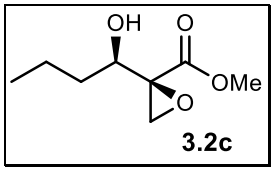
(Yield=167 mg, 99%) (ratio of diastereomers 67/33). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 4.33 (1H, q, *J*=6.6 Hz) (minor), 4.16 (1H, q, *J*=6.4 Hz) (major), 3.71 (3H, s), 3.10 (1H, d, *J*=5.9 Hz) (major), 4.64 (1H, d, *J*=6.1 Hz) (minor), 2.99 (1H, d, *J*=6.0 Hz) (minor), 2.96 (1H, d, *J*=5.8 Hz) (major), 2.08 (1H, br s), 1.31 (3H, d, *J*=6.4 Hz) (major), 1.29 (3H, d, *J*=6.6 Hz) (minor).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 170.0 (minor), 169.9 (major), 65.1 (major), 64.9 (minor), 59.6 (minor), 59.0 (major), 52.5 (major), 52.4 (minor), 49.3 (minor), 49.2 (major), 18.6 (minor), 18.2 (major) ppm. IR (KBr) $\delta$ 3932, 3839, 2984, 2363, 1738, 1519, 1382, 1285, 1173, 1095, 971, 913, 853 cm$^{-1}$. HRMS *m*/*z* calcd for $C_6H_{10}O_4Na$ [M+Na$^+$]: 169.0477, found: 169.0478.

*Methyl 2-(1-hydroxypropyl)oxirane-2-carboxylate* ***3.2b/3.3b***



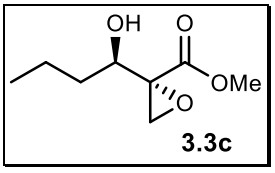

(Yield=128 mg, 70%) (ratio of diastereomers 76/24). $^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 3.75 (1H, m), 3.71 (3H, s), 3.12 (1H, d, $J$=6.0 Hz), 2.98 (1H, d, $J$=6.0 Hz), 2.55 (1H, br s), 1.72 (1H, m), 1.48 (1H, m), 0.98 (3H, t,$J$=6.7 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 170.0, 71.0, 50.1, 52.5, 49.6, 26.0, 9.9 ppm. IR (KBr) $\delta$ 3770, 3457, 2939, 2360, 1869, 1637, 1541, 1440, 1348, 1197, 1139, 1055, 950, 758 cm$^{-1}$. HRMS $m/z$ calcd for $C_7H_{12}O_4Na$ [M+Na$^+$]: 183.0633, found: 183.0636 (Yield **3.2c**/**3.3c**=99%).

*syn-Methyl 2-(1-hydroxybutyl)oxirane-2-carboxylate* ***3.2c***



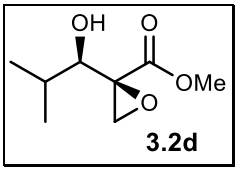

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 3.84–3.87 (1H, m), 3.78 (3H, s), 3.12 (1H, d, $J$=5.9 Hz), 2.98 (1H, d,$J$=5.9 Hz), 1.69–1.40 (4H, m), 0.94 (3H, t, $J$=7.2 Hz).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 170.0, 69.3, 58.3, 52.4, 49.6, 35.0, 18.7, 13.8 ppm. IR (KBr) $\delta$ 3649, 2960, 2361, 1740, 1560, 1457, 1382, 1197, 1139, 1077, 983, 760 cm$^{-1}$. HRMS $m/z$ calcd for $C_8H_{14}O_4Na$ [M+Na$^+$]: 197.0790, found: 197.0786.

*anti-Methyl 2-(1-hydroxybutyl)oxirane-2-carboxylate* ***3.3c***



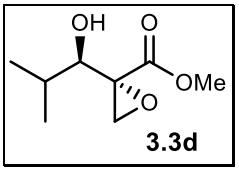

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 4.12–4.10 (1H, m), 3.78 (3H, s), 3.08 (1H, d, *J*=6.0 Hz), 2.98 (1H, d,*J*=6.0 Hz), 1.77 (1H, br s), 1.61–1.37 (4H, m), 0.94 (3H, t, *J*=7.1 Hz).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 170.0, 69.0, 59.4, 52.5, 49.3, 35.1, 18.9, 13.7 ppm. IR (KBr) $\delta$ 3466, 2960, 1739, 1639, 1567, 1441, 1356, 1287, 1212, 1197, 1138, 1129, 1036, 982, 957 cm$^{-1}$ (Yield **3.2d**/**3.3d**=71%).

*syn-Methyl 2-(1-hydroxy-3-methylbutyl)oxirane-2-carboxylate* ***3.2d***



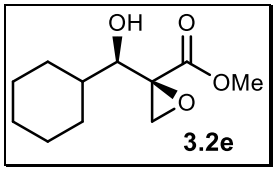

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 3.92 (1H, dd, *J*=3.8, 9.2 Hz), 3.78 (3H, s), 3.12 (1H, d, *J*=5.9 Hz), 2.98 (1H, d,*J*=5.9 Hz), 1.93–1.86 (1H, m), 1.41–1.51 (2H, m), 0.95 (6H, t, *J*=6.5 Hz).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$170.0, 68.0, 58.5, 52.5, 49.6, 41.8, 24.4, 23.5, 21.4 ppm. IR (KBr) $\delta$ 3743, 2956, 2361, 1738, 1438, 1368, 1171, 1116, 1078, 994, 919, 864, 758 cm$^{-1}$. HRMS *m*/*z* calcd for $C_9H_{16}O_4Na$ [M+Na$^{+}$]: 211.0946, found: 211.0942.

*anti-Methyl 2-(1-hydroxy-3-methylbutyl)oxirane-2-carboxylate* ***3.3d***



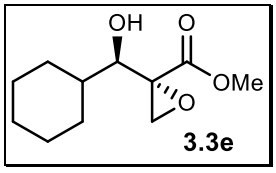
$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 4.20 (1H, dd, $J$=3.8, 9.2 Hz), 3.77 (3H, s), 3.09 (1H, d, $J$=5.9 Hz), 2.98 (1H, d,$J$=5.9 Hz), 2.06–1.96 (1H, br s), 1.76–1.82 (1H, m), 1.48–1.51 (1H, m), 1.27–1.35 (1H, m), 0.95 (6H, t,$J$=6.5 Hz).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 170.0, 67.5, 59.6, 52.5, 49.3, 42.0, 24.4, 23.5, 21.3 ppm. IR (KBr)$\delta$ 3491, 2957, 2393, 1738, 1440, 1368, 1184, 1115, 1094, 993, 919, 879 cm$^{-1}$.

*syn-Methyl 2-(cyclohexyl(hydroxy)methyl)oxirane-2-carboxylate* ***3.2e***



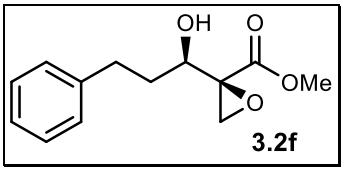

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 3.78 (3H, s), 3.39 (1H, d, $J$=6.7 Hz), 3.11 (1H, d, $J$=5.9 Hz), 2.96 (1H, d,$J$=5.9 Hz), 2.12 (1H, br s), 1.88 (1H, m), 1.75–1.63 (5H, m), 1.26–1.03 (5H, m). $^{13}$C NMR (125 MHz, $CDCl_3$)$\delta$ 170.1, 75.1, 57.3, 52.4, 49.7, 41.2, 29.3, 28.2, 26.2, 26.0, 25.8 ppm. IR (KBr) $\delta$ 3799, 2930, 2669, 2342, 1741, 1377, 1306, 1200, 1124, 1087, 1030, 932, 761 cm$^{-1}$. HRMS $m/z$ calcd for $C_{11}H_{18}O_4Na$ [M+Na$^{+}$]: 237.1103, found: 237.1105.

*anti-Methyl 2-(cyclohexyl(hydroxy)methyl)oxirane-2-carboxylate* ***3.3e***



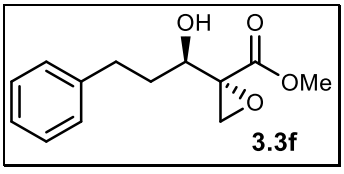

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 3.77 (3H, s), 3.69 (1H, d, $J$=6.5 Hz), 3.02 (1H, d, $J$=5.9 Hz), 2.97 (1H, d,$J$=5.9 Hz), 1.94 (1H, m), 1.78–1.64 (5H, m), 1.30–0.94 (5H, m).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.8, 74.5, 65.7, 58.7, 52.5, 49.6, 41.5, 29.6, 28.2, 26.1, 25.8, 15.1 ppm. IR (KBr) $\delta$ 3752, 2936, 2668, 2341, 1740, 1422, 1232, 1153, 1104, 1069, 1052, 974, 957 cm$^{-1}$.

*syn-Methyl 2-(1-hydroxy-3-phenylpropyl)oxirane-2-carboxylate* ***3.2f***



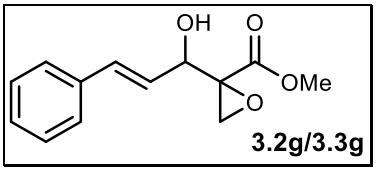

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.33–7.20 (5H, m), 3.95 (1H, m), 3.77 (3H, s), 3.12 (1H, d, *J*=6.0 Hz), 2.95 (1H, d, *J*=6.0 Hz), 2.93–2.88 (1H, m), 2.77–2.65 (1H, m), 2.20–2.01 (1H, m), 1.82–1.93 (1H, m).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 170.0, 141.5, 128.4, 126.1, 68.9, 58.3, 52.6, 49.3, 34.8, 31.7 ppm. IR (KBr) $\delta$ 3873, 3063, 3003, 2924, 2364, 1748, 1290, 1240, 1132, 1075, 754, 701 $cm^{-1}$.

*anti-Methyl 2-(1-hydroxy-3-phenylpropyl)oxirane-2-carboxylate* ***3.3f***



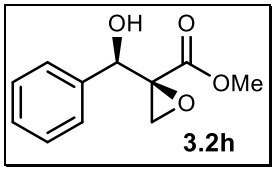

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.30–7.17 (5H, m), 4.11 (1H, d, *J*=9.3 Hz), 3.76 (3H, s), 3.05 (1H, d,*J*=6.0 Hz), 3.00 (1H, d, *J*=6.0 Hz), 2.93–2.87 (1H, m), 2.75–2.69 (1H, m), 1.99–1.93 (1H, m), 1.89–1.82 (1H, m), 1.54 (1H, br s).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 170.0, 141.3, 128.4, 126.0, 68.9, 59.1, 52.3, 49.3, 34.5, 31.8 ppm.

*(*E*)-Methyl 2-(1-hydroxy-3-phenylallyl)oxirane-2-carboxylate* ***3.2g/3.3g***



(Yield=103 mg, 47%) (ratio of diastereomers 53/47). $^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.40–7.24 (m, 5H), 6.74 (1H, d, $J$=16.0 Hz) (major and minor), 6.27 (1H, dd, $J$=6.3, 12.0 Hz) (major), 6.23 (1H, dd, $J$=5.8, 13.2 Hz) (minor), 4.85 (1H, dd, $J$=6.3, 1.3 Hz) (minor), 4.71 (1H, dd, $J$=6.5, 1.2 Hz) (major), 3.80 (3H, s) (major), 3.79 (3H, s) (minor), 3.15 (1H, d, $J$=5.9 Hz) (major), 3.13 (1H, d, $J$=6.1 Hz) (minor), 3.06 (1H, d, $J$=6.1 Hz) (minor), 3.00 (1H, d, $J$=5.9 Hz) (major). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.8 (minor), 169.7 (major), 136.2 (minor), 136.1 (major), 133.5 (major), 133.1 (minor), 128.6, 128.1, 128.0, 126.7 (major and minor), 125.9 (minor), 125.6 (major), 70.7 (major), 70.0 (minor), 59.0 (minor), 58.5 (major), 52.7, 52.6 (major and minor), 49.9 (major), 49.2 (minor) ppm. HRMS $m/z$ calcd for $C_{13}H_{14}O_4Na$ [M+Na$^+$]: 257.0790, found: 257.0792.

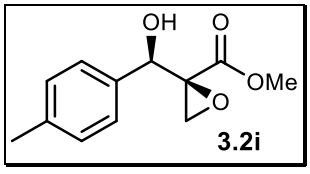
*syn-Methyl 2-(hydroxy(phenyl)methyl)oxirane-2-carboxylate* ***3.2h*** [10]



**3.2h**

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.43–7.30 (5H, m), 5.18 (1H, s), 3.73 (3H, s), 3.12 (1H, d, $J$=5.9 Hz), 2.86 (1H, d, $J$=5.9 Hz).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.7, 138.4, 128.4, 127.0, 71.7, 59.0, 52.6, 49.7 ppm. IR (KBr) $\delta$ 3487, 3064, 2910, 2359, 1739, 1269, 1160, 1082, 1027, 947, 757 cm$^{-1}$. HRMS $m/z$ calcd for $C_{11}H_{12}O_4Na$ [M+Na$^+$]: 231.0633, found: 231.0632.

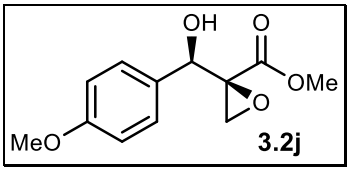
*syn-Methyl 2-(hydroxy(*p*-tolyl)methyl)oxirane-2-carboxylate* ***3.2i***



**3.2i**

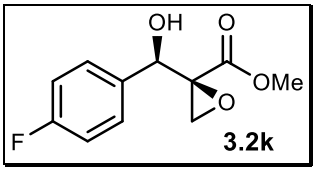
$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.29 (2H, d, $J$=8.0 Hz), 7.26 (2H, d, $J$=8.0 Hz), 5.15 (1H, s), 3.72 (3H, s), 3.11 (1H, d, $J$=5.9 Hz), 2.86 (1H, d, $J$=5.9 Hz).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.8, 137.9, 135.5, 128.9, 127.0, 71.4, 59.1, 52.6, 49.6, 21.1 ppm. IR (KBr) $\delta$ 3502, 3005, 2923, 1743, 1197, 1125, 1020, 943, 837, 765, 686 cm$^{-1}$. HRMS *m/z* calcd for $C_{12}H_{14}O_4Na$ [M+Na$^+$]: 245.0790, found: 245.0787.

*syn-Methyl 2-(hydroxy(4-methoxyphenyl)methyl)oxirane-2-carboxylate* ***3.2j***



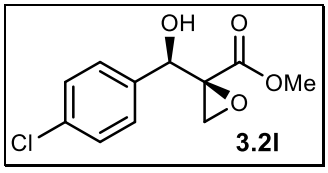

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.33 (2H, d, $J$=8.8 Hz), 6.87 (2H, d, $J$=8.8 Hz), 5.16 (1H, s), 3.79 (3H, s), 3.67 (3H, s), 3.12 (1H, d, $J$=6.0 Hz), 2.85 (1H, d, $J$=6.0 Hz).$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.8, 159.5, 130.4, 128.5, 113.8, 71.2, 59.1, 55.2, 52.6, 49.5 ppm. IR (KBr) $\delta$ 3493, 3003, 2910, 1742, 1197, 1124, 1031, 978, 917, 836, 756 cm$^{-1}$. HRMS *m/z* calcd for $C_{12}H_{14}O_5Na$ [M+Na$^+$]: 261.0739, found: 261.0738.

*syn-Methyl 2-((4-fluorophenyl)(hydroxy)methyl)oxirane-2-carboxylate* ***3.2k***



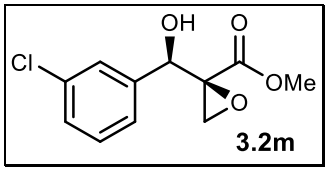

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.40 (2H, dd, $J$=8.5, 5.5 Hz), 7.03 (2H, t, $J$=8.7 Hz), 5.15 (1H, s), 3.73 (3H, s), 3.13 (1H, d, $J$=6.0 Hz), 2.85 (1H, d, $J$=6.0 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.7, 162.2 (d, $J$=245 Hz), 134.2, 129.0 (dd, $J$=7.2, 21.3 Hz), 115.2 (dd, $J$=12.5, 22.5 Hz), 71.1, 65.8, 52.7, 49.6 ppm. IR (KBr) $\delta$ 3477, 3070, 2958, 2342, 1737, 1509, 1398, 1271, 1197, 1128, 1045, 980, 842, 756 cm$^{-1}$. HRMS *m/z* calcd for $C_{11}H_{11}FO_4Na$ [M+Na$^+$]: 249.0539, found: 249.0535.

*syn-Methyl 2-((4-chlorophenyl)(hydroxy)methyl)oxirane-2-carboxylate* ***3.2l***



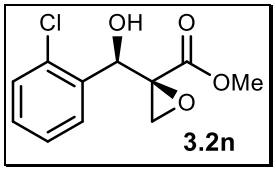

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.37 (2H, d, *J*=8.0 Hz), 7.32 (2H, d, *J*=8.0 Hz), 5.29 (1H, s), 3.73 (3H, s), 3.14 (1H, d, *J*=6.0 Hz), 2.88 (1H, d, *J*=6.0 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.6, 137.0, 134.1, 128.6, 128.5, 71.2, 58.7, 52.7, 49.7 ppm. IR (KBr) $\delta$ 3518, 3001, 2929, 1723, 1411, 1287, 1160, 1107, 1049, 982, 920, 756 cm$^{-1}$. HRMS *m*/*z* calcd for $C_{11}H_{11}ClO_4Na$ [M+Na$^+$]: 265.0244, found: 265.0245.

*syn-Methyl 2-((3-chlorophenyl)(hydroxy)methyl)oxirane-2-carboxylate* ***3.2m***



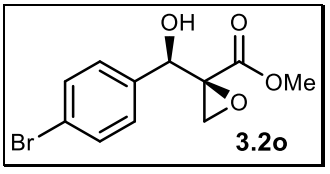

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.44 (1H, s), 7.32 (3H, m), 5.10 (1H, s), 3.73 (3H, s), 3.16 (1H, d, *J*=6.0 Hz), 2.90 (1H, d, *J*=6.0 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.6, 140.6, 134.3, 129.6, 128.4, 127.1, 125.3, 71.3, 58.7, 52.8, 49.8 ppm. IR (KBr) $\delta$ 3466, 3020, 2964, 1736, 1463, 1264, 1154, 1170, 1083, 962, 918, 877 cm$^{-1}$.

*syn-Methyl 2-((2-chlorophenyl)(hydroxy)methyl)oxirane-2-carboxylate* ***3.2n***



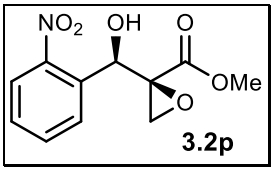
$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.54 (1H, m), 7.33 (1H, m), 7.24–7.29 (2H, m), 6.04 (1H, s), 3.82 (3H, s), 3.06 (1H, d, $J$=6.0 Hz), 2.35 (1H, d, $J$=6.0 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.9, 135.0, 132.9, 129.5, 127.9, 126.9, 67.9, 58.6, 52.9, 50.5 ppm. IR (KBr) $\delta$ 3741, 3019, 2938, 1734, 1472, 1390, 1297, 1195, 1064, 1028, 1028, 758, 741 cm$^{-1}$.

*syn-Methyl 2-((4-bromophenyl)(hydroxy)methyl)oxirane-2-carboxylate* ***3.2o***



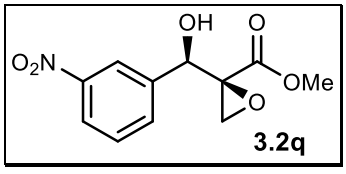

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.48 (2H, d, $J$=8.0 Hz), 7.32 (2H, d, $J$=8.0 Hz), 5.29 (1H, s), 3.73 (3H, s), 3.14 (1H, d, $J$=6.0 Hz), 2.88 (1H, d, $J$=6.0 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.6, 137.5, 131.5, 129.0, 122.2, 71.2, 58.7, 52.7, 48.9 ppm. IR (KBr) $\delta$ 3711, 3077, 2957, 2360, 1923, 1592, 1728, 1460, 1333, 1286, 1196, 1127, 1049, 935, 755 cm$^{-1}$. HRMS $m/z$ calcd for $C_{11}H_{11}BrO_4Na$ [M+Na$^+$]: 308.9738, found: 308.9735.

*syn-Methyl 2-((2-nitrophenyl)(hydroxy)methyl)oxirane-2-carboxylate* ***3.2p***



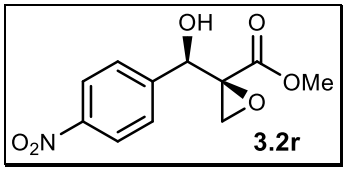

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.96 (1H, dd, $J$=8.2, 1.3 Hz), 7.79 (1H, dd, $J$=7.9, 1.3 Hz), 7.65 (1H, td, $J$=7.7, 1.3 Hz), 7.48 (1H, td, $J$=8.5, 1.4 Hz), 6.17 (1H, s), 3.82 (3H, s), 3.13 (1H, d, $J$=6.0 Hz), 2.37 (1H, d,$J$=6.0 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.6, 148.3, 133.3, 129.8, 129.1, 124.6, 66.8, 58.1, 53.1, 51.0 ppm. IR (KBr) $\delta$ 3648, 3093, 2957, 1725, 1440, 1357, 1267, 1200, 1156, 1053, 947, 747 cm$^{-1}$.

*syn-Methyl 2-((3-nitrophenyl)(hydroxy)methyl)oxirane-2-carboxylate* ***3.2q***



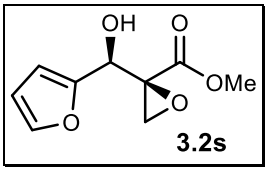

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.33 (1H, m), 8.17 (1H, ddd, *J*=8.2, 2.3, 1.2 Hz), 7.81 (1H, m), 7.52 (1H, t,*J*=7.92 Hz), 5.19 (1H, s), 3.74 (3H, s), 3.22 (1H, d, *J*=6.0 Hz), 2.95 (1H, d, *J*=6.0 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.4, 148.2, 133.3, 129.2, 123.2, 122.1, 71.0, 65.7, 52.8, 49.7 ppm. IR (KBr) $\delta$ 3712, 3092, 3006, 2957, 2876, 1735, 1441, 1353, 1289, 1163, 1096, 976, 935, 866, 758 $cm^{-1}$.

*syn-Methyl 2-((4-nitrophenyl)(hydroxy)methyl)oxirane-2-carboxylate* ***3.2r***




$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.22 (2H, d, *J*=8.0 Hz), 7.65 (2H, d, *J*=8.0 Hz), 5.13 (1H, s), 3.73 (3H, s), 3.48 (1H, d, *J*=6.0 Hz), 2.94 (1H, d, *J*=6.0 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.5, 146.0, 128.1, 127.8, 123.4, 71.4, 58.4, 53.0, 49.9 ppm. IR (KBr) $\delta$ 3902, 3087, 2958, 2342, 1925, 1715, 1517, 1442, 1221, 1096, 1053, 946, 777 $cm^{-1}$. HRMS *m/z* calcd for $C_{11}H_{11}NO_6Na$ $[M+Na^+]$: 276.0484, found: 276.0482.

*syn-Methyl 2-(furan-2-yl(hydroxy)methyl)oxirane-2-carboxylate* ***3.2s***

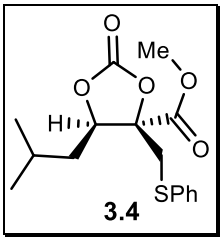


$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.38 (1H, s), 7.26 (1H, s), 6.39 (1H, m), 6.33 (1H, m), 5.29 (1H, s), 3.75 (3H, s), 3.23 (1H, d, $J$=6.0 Hz), 3.05 (1H, d, $J$=6.0 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.3, 125.6, 142.5, 110.4, 107.7, 64.5, 52.6, 49.0 ppm. IR (KBr) $\delta$ 3932, 3153, 3004, 2957, 1734, 1633, 1359, 1231, 1048, 975, 753 $cm^{-1}$.

General experimental procedure for the preparation of cyclic carbonates (**3.4-3.7**)

An ice-bath cold suspension of sodium hydride (60% in mineral oil) (1.12 mmol) in THF (1 mL) was treated with thiophenol (2.25 mmol). The mixture was stirred at rt for 15 min and then a solution of the epoxyester **3.2** (0.75 mmol) in THF (1 mL) was added drop wise and the mixture was stirred at rt for 1.5 h. Then it was treated with pyridine (0.22 mmol) and triphosgene (0.48 mmol). The mixture was refluxed for 15 h. Then brine was added and extracted with $Et_2O$ (3×20 mL), the organic layers were washed (brine), dried ($Na_2SO_4$), and concentrated. The crude oil was purified through chromatography (silica-gel, hexanes/EtOAc (8:2) and (7:3)).

*syn-Methyl-5-isobutyl-2-oxo-4-((phenylthio)methyl)-1,3-dioxolane-4-arboxylate **3.4***

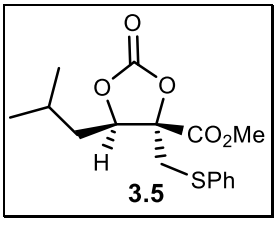


**3.4**

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.46 (2H, m), 7.25–7.33 (3H, m), 4.73 (1H, m), 3.81 (3H, s), 3.58 (1H, d,$J$=15.0 Hz), 3.47 (1H, d, $J$=15.0 Hz), 1.77 (1H, m), 1.44 (1H, m),

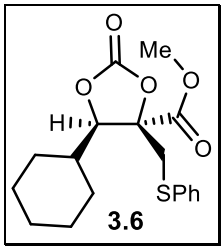
1.35 (1H, m), 0.92 (3H, d, *J*=6.5 Hz), 0.84 (3H, d, *J*=6.5 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 167.7, 152.7, 134.7, 131.3, 129.3, 127.7, 86.6, 80.4, 53.2, 39.6, 38.6, 25.1, 23.0, 21.2 ppm. IR (KBr) $\delta$ 3059, 2959, 1811, 1743, 1626, 1540, 1470, 1387, 1306, 1200, 1116, 1025, 968, 746 cm$^{-1}$. HRMS *m*/*z* calcd for $C_{16}H_{20}O_5SNa$ [M+Na$^+$]: 347.0929, found: 347.0929.

*anti-Methyl-5-isobutyl-2-oxo-4-((phenylthio)methyl)-1,3-dioxolane-4-carboxylate* ***3.5***




$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.46 (2H, m), 7.25–7.33 (3H, m), 4.76 (1H, m), 3.70 (3H, s), 3.42 (2H, s), 1.71 (1H, m), 1.49 (1H, m), 1.47 (1H, m), 0.98 (3H, d, *J*=6.5 Hz), 0.95 (3H, d, *J*=6.5 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 168.6, 152.2, 134.4, 131.9, 129.2, 127.8, 85.6, 80.4, 53.4, 37.9, 37.5, 24.9, 23.2, 21.2 ppm. IR (KBr) $\delta$ 3059, 2959, 1806, 1749, 1582, 1439, 1360, 1257, 1132, 1048, 963, 744 cm$^{-1}$.

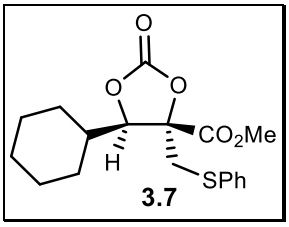
*syn-Methyl-5-cyclohexyl-2-oxo-4-((phenylthio)methyl)-1,3-dioxolane-4-carboxylate* ***3.6***




$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.39 (2H, m), 7.25–7.17 (3H, m), 4.37 (1H, m), 3.73 (3H, s), 3.57 (1H, d,*J*=15.0 Hz), 3.35 (1H, d, *J*=15.0 Hz), 1.80 (1H, m), 1.40–1.77 (5H, m), 1.24–0.79 (5H, m). $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 168.9, 152.4, 134.8,

131.9, 129.2, 127.8, 86.0, 85.7, 53.5, 37.7, 37.3, 29.5, 28.1, 25.7, 25.4, 25.1 ppm. IR (KBr) $\delta$ 3060, 2929, 2857, 1741, 1582, 1402, 1195, 1024, 927, 845, 713, 629 $cm^{-1}$. HRMS *m*/*z* calcd for $C_{18}H_{22}O_5SNa$ [$M+Na^+$]: 373.1086, found: 373.1089.

*anti-Methyl-5-cyclohexyl-2-oxo-4-((phenylthio)methyl)-1,3-dioxolane-4-carboxylate* ***3.7***



**3.7**

$^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 7.47 (2H, m), 7.24–7.33 (3H, m), 4.43 (1H, m), 3.76 (3H, s), 3.56 (1H, d,*J*=15.0 Hz), 3.48 (1H, d, *J*=15.0 Hz), 1.77 (1H, m), 1.68–1.76 (5H, m), 1.10–1.25 (5H, m). $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$ 167.9, 152.6, 134.7, 131.7, 129.2, 127.7, 86.6, 85.8, 53.3, 40.8, 38.6, 28.6, 28.5, 25.7, 25.2, 25.0 ppm. IR (KBr) $\delta$ 2934, 2854, 1747, 1584, 1584, 1440, 1178, 1052, 930, 634 $cm^{-1}$.

# Chapter 4:

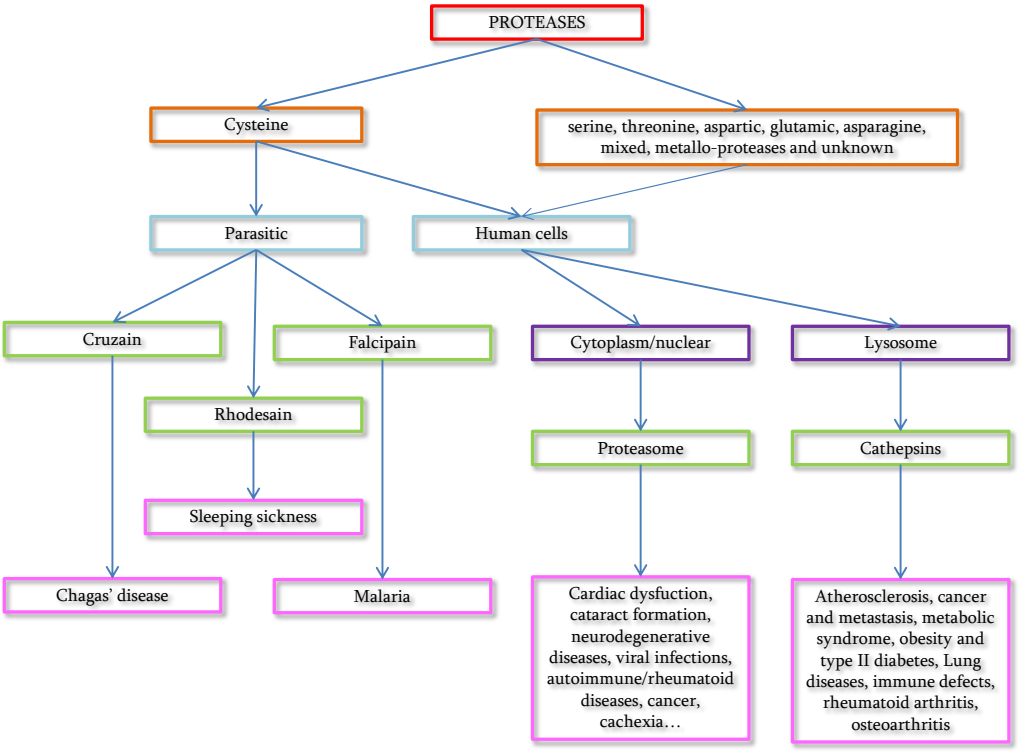
# Design, synthesis and evaluation of new inhibitors of cysteine proteases and proteasome

# 1. Introduction

## 1.1. Proteases

Proteases are a big family of enzymes with proteolytic activity, catalyzing the hydrolysis of peptides bonds. Proteases are involved in numerous important physiological processes including protein turnover, digestion, blood coagulation and wound healing, fertilization, cell differentiation and growth, cell signaling, the immune response, and apoptosis. The loss of the control in this activity causes some diseases such as Alzheimer's disease, stroke, cancer, emphysema, inflammation, viral infections and cataracts.

For better understanding of subsequent parts of the chapter a scheme has been represented in **scheme 1**.

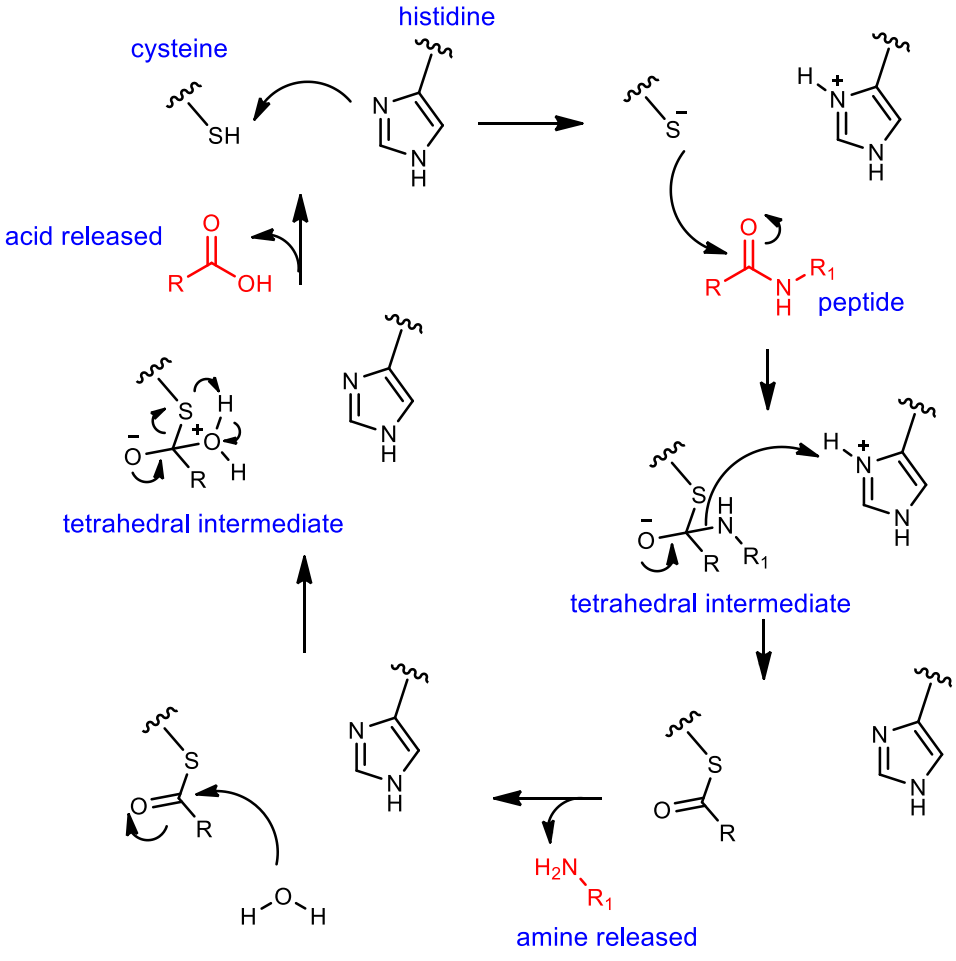



**Scheme 1.** General proteases scheme

## 1.2. Cysteine proteases

Cysteine proteases are a big group of proteases, all having a cysteine residue in the active site causing the proteolysis. General mechanism is depicted in **scheme 2**. In the first step a deprotonation of the sulfhydryl group by histidine residue takes place, forming a dyad (other cysteine proteases form a triad with an extra amino acid). Next step involves a nucleophilic attack of the thiolate anion to the carbonyl carbon of the peptide bond. A tetrahedral intermediate is formed and oxyanion is stabilized by hydrogen bond forming backbones with other amides. Then, the acylation of the enzyme occurs releasing the amino group of the C-terminal part of the peptide. A hydrolysis of the thioester leads to other tetrahedral

intermediate, collapsing to release the acid group of the N-terminal part of the peptide, regenerating the free enzyme.




**Scheme 2.** Cysteine protease general mechanism

### 1.2.1. Classification of cysteine proteases

Cysteine proteases are classified into 14 clans members based on the catalytic site composition, mechanism and evolution. The most important family is the CA or papain like, so called for the isolation and characterization of papain from papaya (*Carica papaya*) in 1879. Papain was also the first cysteine protease structure to be solved.

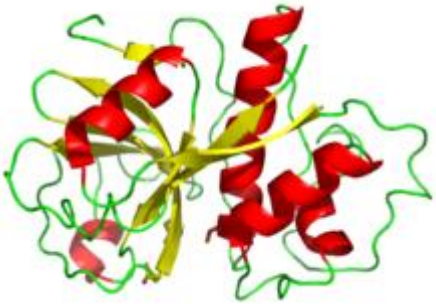


**Figure 1**. Papain structure

On the other hand, cysteine proteases are present in other organisms such as in bacteria, fungi, protozoa (cruzain in *Trypanosoma cruzi*, rhodesain in *Trypanosoma brucei*, amoebopain in *Entamoeba histolytica*), plants and mammals (calpains and cathepsins).

## 1.3. Human proteases

Proteases can mainly develop the proteins degradation in two places of the cell, in cytoplasm/nuclear (pH aprox 7.4) called proteasome or in lysosomes (pH 4 - 5.5) generally cathepsins families.

### 1.3.1. Proteasome: active site, process and diseases

Proteasome recycles the unneeded or damage proteins, with previously mentioned proteases mechanism. The protein, to be degraded, must be tagged to small molecules called ubiquitin. Then the degradation process takes places in a hidden active site of a cylindrical core, illustrated in **figure 2**.

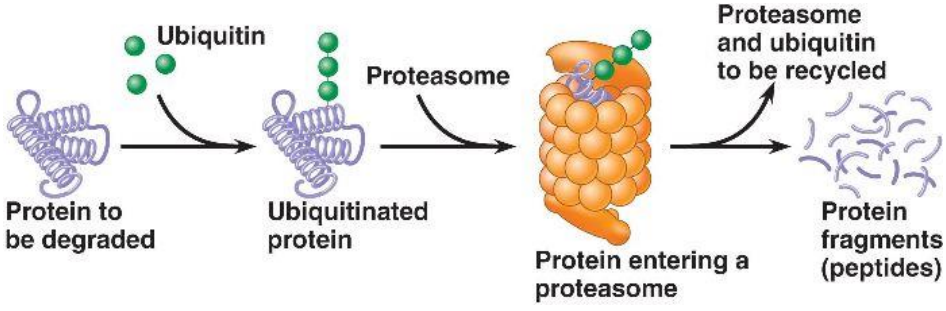



**Figure 2.** Protein degradation process

The proteasome components are referred to Svedberg sedimentation coefficient (S) being the most exclusively in mammals the 26S proteasome. This 26S proteasome contains one 20S protein subunit and two 19S subunits. **Figure 3** shows the scheme of the described distribution and a real lateral and top view of the proteasome.

20S is formed by four heptameric rings, with two different subunits α and β. The main catalytic activity is located in the β-subunits. In eukaryotic proteasomes, containing seven subunits and in the case of mammals only $\beta_1$, $\beta_2$ and $\beta_5$ are catalytic (constitutive proteasome, in brain). On the other hand, the altered proteasome (immunoproteasome, in spleen) with more active sites are described by $\beta_{1i}$, $\beta_{2i}$ and $\beta_{5i}$.

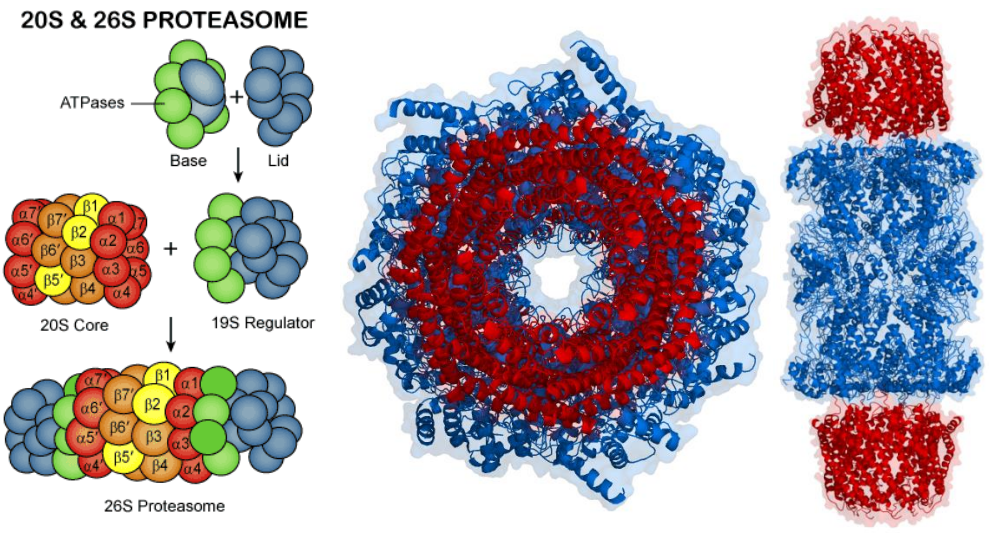



**Figure 3**. Proteasome components

The degradation involved several proteases, depending of the type of the cell, the nature of the process, the place or the unit and other factors but the most important residue is threonine.

The most common diseases related with the non-natural function of these proteasomic enzymes are related to cardiac dysfunctions, cataracts formation, neurodegenerative disorders (Parkinson, Alzheimer, Huttington…), viral infections (HIV…), autoimmune diseases and cancer.

### 1.3.2. Human cathepsins

In the lysosomal system, the degradation of proteins is normally carried out by a combination of several proteases, amylases, lipases and nucleases. The most important known proteases are aspartic, serine and cysteine protease. Cysteine

cathepsins are essential in most lysosomal proteases and these cathepsins belong to the papain family of cysteine proteases.

Most of cathepsins are from lysosomal cysteine proteases (B, C, F, H, K, L, O, S, V, X and W) and the rest are from aspartic proteases (D and E) or serine (A and G).

Disorder or abnormal function, **figure 4**, of these cathepsins (overexpression or modified activity) caused several diseases in the organism as atherosclerosis, cancer and metastasis, metabolic syndrome, obesity and type II diabetes, Lung diseases, immune defects, rheumatoid arthritis, osteoarthritis.

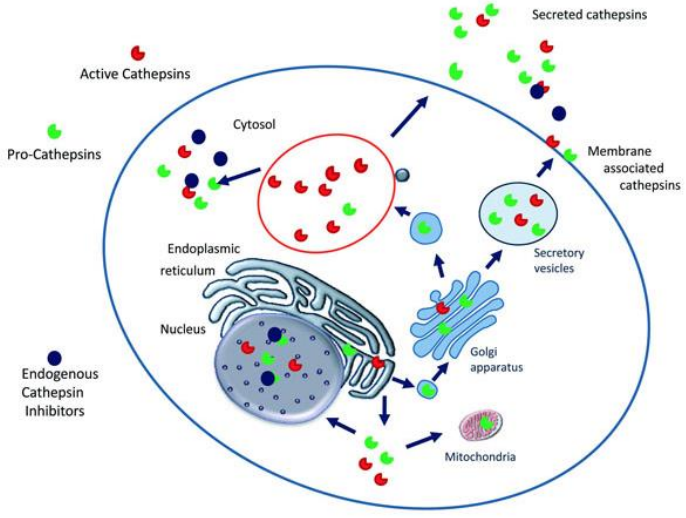



**Figure 4.** Cellular location of cathepsins

## 1.4. Parasitic diseases

The presence of these proteases in viruses and other organisms denotes the importance of these enzymes in the cycle life of the some parasites. For this reason

the study of these enzymes and their inhibition could be a good strategy to compete and to cure the diseases provoked.

### 1.4.1. American trypanosomiasis (Chagas' disease)

Chagas' disease is caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). It is located in Latin American in areas from 21 countries. According to the last WHO study in 2014, is calculated over 7-8 million people are infected.

*T. cruzi* parasites are mainly transmitted by contact with the faeces of infected blood-sucking triatomine bugs. These bugs, vectors that carry the parasites, typically live in the cracks of poorly-constructed homes in rural or suburban areas. Normally they are hidden during the day and become active at night, when they feed on human blood. They usually bite an exposed area of skin such as the face, and the bug defecates close to the bite. The parasites enter into the body when the person instinctively scrubs the bug faeces into the bite, the eyes, the mouth, or into any skin break.

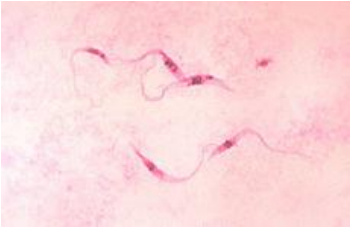
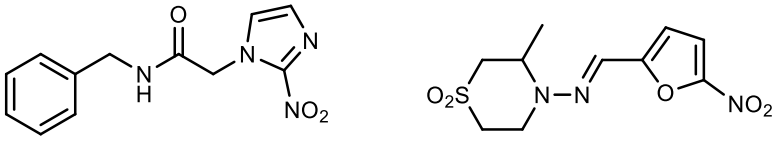
| Vector | Parasite |
| --- | --- |
|  |  |
| *Triatominae* | *Trypanosoma cruzi* |

**Figure 5**. Vector and parasite for Chagas' disease

Chagas' disease can be treated with Benznidazole and also Nifurtimox. Both medicines are almost 100 % effective in curing the disease if given soon after infection at the onset of the acute phase. However, the efficacy of both diminishes the longer a person has been infected. Treatment is also indicated for those in whom the infection has been reactivated (for example due to immunosuppression), for infants with congenital infection and for patients during the early chronic phase. To the infected adults, especially those with no symptoms should be offered the treatment. The potential benefits of medication in preventing or delaying the development of Chagas' disease should be weighed against the long duration of treatment (up to 2 months) and possible adverse reactions (occurring in up to 40 % of treated patients).

The structure of Benznidazole and Nifurtimox is shown in **figure 6**. They should not be taken by pregnant women or by people with kidney or liver failure. Nifurtimox is also contraindicated for people with a background of neurological or psychiatric disorders.




Benznidazole Nifurtimox

**Figure 6**. Structure of Chagas' disease pharmaceuticals

### 1.4.2. African trypanosomiasis (sleeping sickness)

Sleeping sickness is a vector-borne parasitic disease. It is caused by infection with protozoan parasites belonging to the genus *Trypanosoma (Trypanosoma brucei gambiense* is found in 24 countries in west and central Africa

and *Trypanosoma brucei rhodesiense* is found in 13 countries eastern and southern Africa). They are transmitted to humans by tse-tse fly (*Glossina* genus) which have acquired their infection from human beings or from animals harbouring the human pathogenic parasites.

Tse-tse flies are found just in sub-Saharan Africa though only certain species transmit the disease. For reasons that are so far unexplained, there are many regions where tse-tse flies are found, but sleeping sickness is not. Rural populations living in regions where transmission occurs and which depend on agriculture, fishing, animal husbandry or hunting are the most exposed to the tsetse fly and therefore to the disease. The disease develops in areas ranging from a single village to an entire region. Within an infected area, the intensity of the disease can vary from one village to the next.

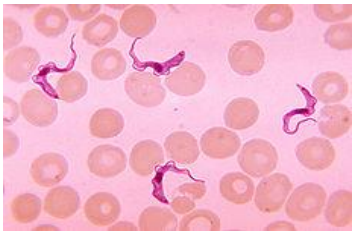
| Vector | Parasite |
| --- | --- |
|  |  |
| *Tse-tse fly* | *Trypanosoma* |

**Figure 7**. Vector and parasite for sleeping sickness

The type of treatment depends on the stage of the disease. The drugs used in the first stage of the disease are of lower toxicity and easier to administer. The earlier the disease is identified, the better the prospect of a cure. In the first stage

Pentamidine and Suramin are used, and in the second stage Melarsoprol, Eflornithine and a combination treatment of Nifurtimox and Eflornithine were usually used, illustrated in **figure 8**.

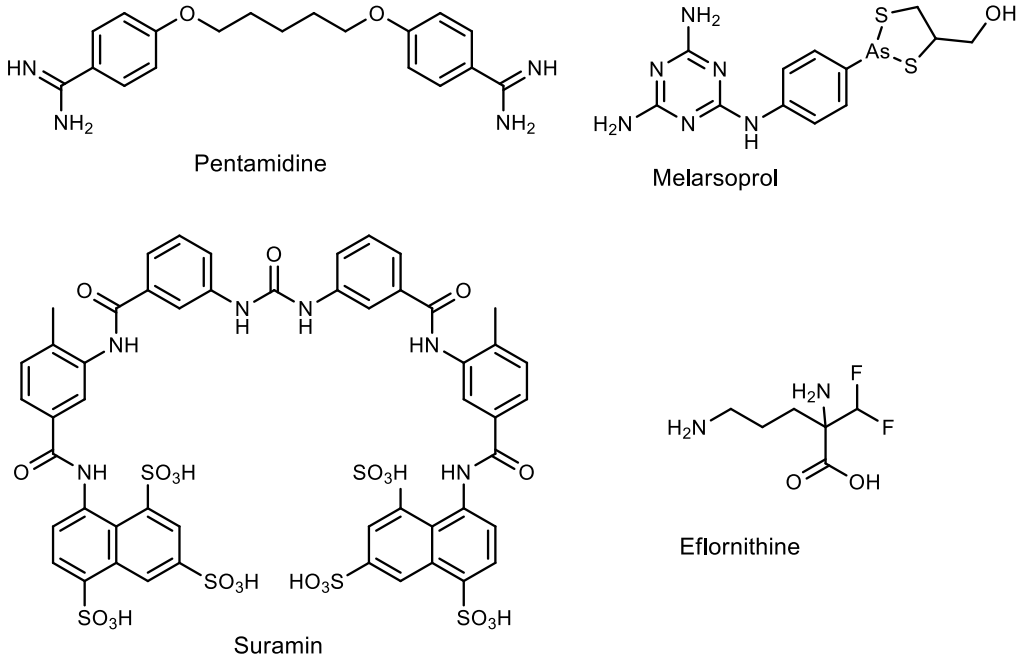



**Figure 8**. Structure of sleeping sickness pharmaceuticals

### 1.4.3. Malaria

Malaria is a mosquito-borne infectious disease; the disease is transmitted by the bite of an infected female *Anopheles* mosquito. This bite introduces the parasites from the mosquito's saliva into a person's blood. Five species of *Plasmodium* can infect and be spread by humans. Most deaths are caused by

*P. falciparum* because *P. vivax*, *P. ovale*, and *P. malariae* generally cause a milder form of malaria.

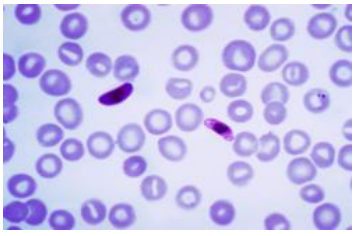
| Vector | Parasite |
| --- | --- |
|  |  |
| *Anopheles stephensi* | *Plasmodium falciparum* |

**Figure 9**. Vector and parasite for malaria

Globally, an estimated 3.2 billion people are at risk of being infected with malaria and developing disease, and 1.2 billion are at high risk. According to the latest estimates for WHO in 2014, 198 million cases of malaria occurred globally in 2013 (uncertainty range 124–283 million) and the disease led to 584000 deaths (uncertainty range 367000–755000). The burden is heaviest in the WHO African Region, where an estimated 90% of all malaria deaths occur, and in children aged less than 5 years, who account for 78 % of all deaths. Several campaigns have been made for many years for these diseases do not been neglected.


**Figure 10**. Neglected diseases campaigns

There are a number of drugs that can help prevent malaria such as Chloroquine and Mefloquine, but cause side effects and there is not one that assures the total protection.

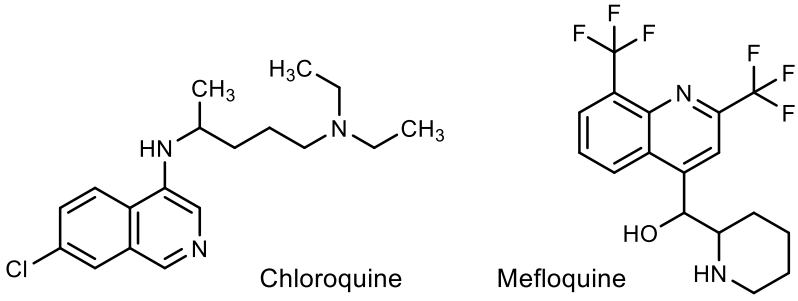



**Figure 11**. Structure of malaria pharmaceuticals

## 1.5. Cysteine protease inhibitors

An inhibitor is a compound that decreases the activity of an enzyme. As previously demonstrated, cysteine proteases are important therapeutic targets in a

large number of diseases; a strategy to remove or decrease the effect of these diseases is based on the design and synthesis of new cysteine proteases inhibitors.

Depending of the nature of the bonding of inhibitor to enzyme in the active site, the inhibition could be reversible or irreversible. In the inhibitors designed in this thesis we focused in the interaction of the inhibitor and the thiol of the cysteine 25 of the active site represented in **figure 12**.

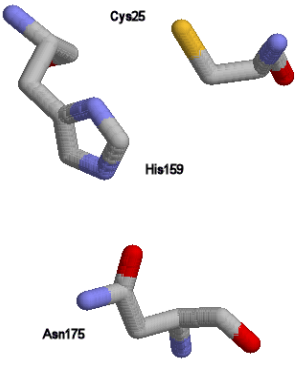
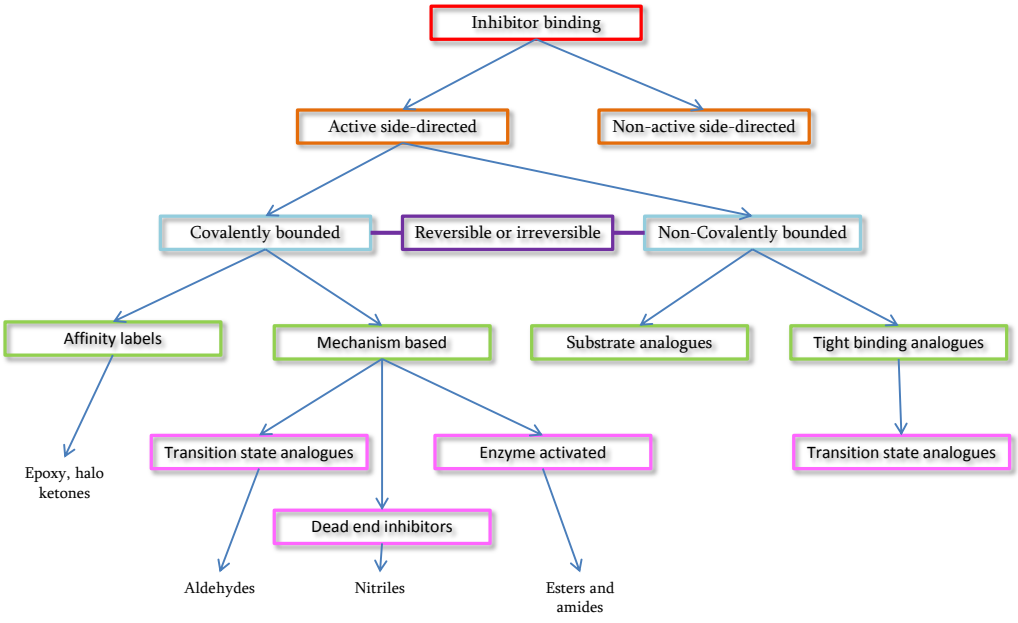



**Figure 12**: Representative active site of cysteine protease

## 1.6. Classification of cysteine protease inhibitors

Completing the previous classification based on the kinetics (reversible or irreversible), further classifications can be done, represented in **scheme 3**. The non-active side directed inhibitors are commonly allosteric effectors, interacting in other place of the enzyme. The active side directed inhibitors, can be divided based on the covalent or non-covalent bonding. The non-covalent bounded are usually substrate analogues or transition state analogues (depending on the similarity to

the catalytic intermediates). The covalent bonding inhibitors are subdivided in transition state analogues (e.g. aldehydes), enzyme activated inhibitors (intermediate goes through a non-catalytic pathway e.g. esters or amides), dead end inhibitors (form irreversible enzyme-inhibitor complex e.g. nitriles) and affinity labels (the irreversible reaction forms permanent enzyme-inhibitor complex e.g. epoxide)



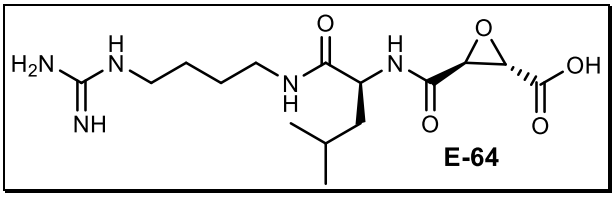

**Scheme 3**. Inhibitor classification

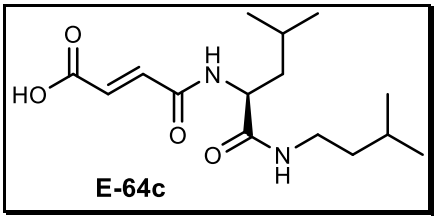
## 1.7. Types of inhibitors[70]

A literature background of the types of inhibitors was done in order to design new compounds with high activity. The attention was focused on epoxides, halomethyl ketones, aziridines and Michael acceptors as the main inhibitors to be analyzed.

### 1.7.1. Epoxides

One of the first cysteine proteases inhibitors was E-64, **figure 13**, an irreversible compound isolated in 1978 from the funghi *Aspergillius japonicus* by Hanada.[71]




[70] Based on: Powers, J. C.; Asgian, J. L.; Ekici, Ö. D.; James, K. E. *Chem. Rev.* **2002**, 102, 4639–4750
[71] Hanada K, Tamai M, Yamagishi M, Ohmura S, Sawada J, Tanaka I. *Agric. Biol. Chem* **1978**, 42, 523–528

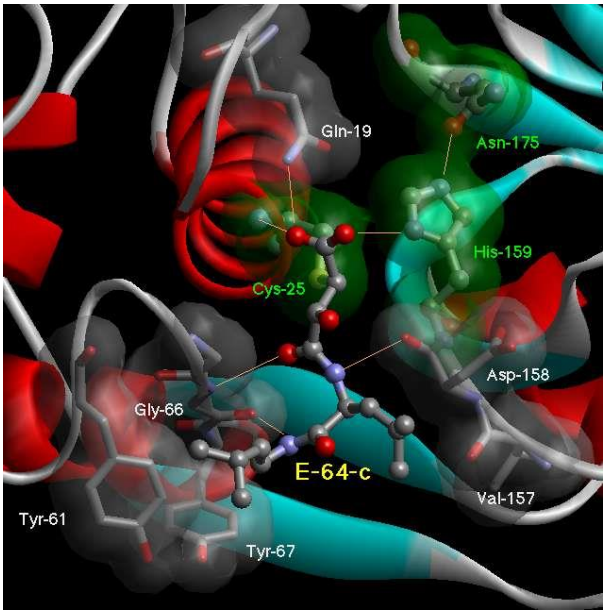



**Figure 13**. E-64c structure and interactions in active site

E-64 inhibits papain, ficin, bromelain, cathepsins B,[72,73] cathepsins H,[74] cathepsins F,[75] cathepsins K,[76,77] cathepsins L,[78,79] cathepsins O,[80] cathepsins S,[81] cathepsins V,[82] and cathepsins X,[83] calpain,[84,85] calpain II,[86] cruzain,[87] and their

[72] Inaba, T.; Hirayama, Y.; Fujinaga, N. *Agric. Biol. Chem.* **1979**, *43*, 655
[73] Hashida, S.; Towatari, T.; Kominami, E.; Katunuma, N. *J. Biochem.* **1980**, *88*, 1805
[74] Barret, J.; Kembhavi,A.; Brown, M.; Kirschke, H.; Knight, C.; Tamai, M.; Hanada, K. *Biochem. J.* **1982**, 201, 189-198
[75] Wang, B.; Shi, G. P.; Yao, P. M.; Li, Z.; Chapman, H. A.; Bromme, D. *J. Biol. Chem.* **1998**, *273*, 32000
[76] Bossard, M. J.; Tomaszek, T. A.; Thompson, S. K.; Amegadzie, B. Y.; Hanning, C. R.; Jones, C.; Kurdyla, J. T.; McNulty, D. E.; Drake, F. H.; Gowen, M.; Levy, M. A. *J. Biol. Chem.* **1996**, *271*, 12517
[77] Aibe, K.; Yazawa, H.; Abe, K.; Teramura, K.; Kumegawa, M.; Kawashima, H.; Honda, K. *Biol. Pharm. Bull.* **1996**, *19*, 1026
[78] Towatari, T.; Tanaka, K.; Yoshikawa, D.; Katunuma, N. *J. Biochem.* **1978**, *84*, 659
[79] Katunuma, N.; Towatari, T.; Kominami, E.; Hashida, S.; Takio, K.; Titani, K. *Acta Biol. Med. Ger.* **1981**, *40*, 1419
[80] Santamaria, I.; Velasco, G.; Pendas, A. M.; Paz, A.; Lopez-Otin, C. *J. Biol. Chem.* **1999**, *274*, 13800
[81] Goursalin, B. J.; Lachance, P.; Bonneau, P. R.; Storer, A. C.; Kirschke, H.; Bro¨mme, D. *Bioorganic Chem.* **1994**, *22*, 227
[82] Bro¨mme, D.; Li, Z.; Barnes, M.; Mehler, E. *Biochemistry* **1999**, *38*, 2377
[83] Therrien, C.; Lachance, P.; Sulea, T.; Purisima, E. O.; Qi, H. T.; Ziomek, E.; Alvarez-Hernandez, A.; Roush, W. R.; Menard, R. *Biochemistry* **2001**, *40*, 2702
[84] Sugita, H.; Ishiura, S.; Suzuki, K.; Imahori, K. *J. Biochem. (Tokyo),* **1980**, *87*, 339.
[85] Suzuki, K. *J. Biochem. (Tokyo)* **1983**, *93*, 1305
[86] Parkes, C.; Kembhavi, A. A.; Barrett, A. J. *Biochem. J.* **1985**, *230*, 509
[87] Roush, W. R.; Hernandez, A. A.; McKerrow, J. H.; Selzer, P. M.; Hansell, E.; Engel, J. C. *Tetrahedron* **2000**, *56*, 9747

crystal structure in papain.[88] Several research groups have synthetized modified analogs to increase the inhibition results.

In our research group, we explored new dipeptidyl epoxide compounds as a new warheads based on previously reported epoxyketones.[89] This new generation was called α,β-epoxyesters (**figure 14**) and they were very potent irreversible inhibitors of cysteine proteases, as it is shown in **table 1** with E-64c.[90]

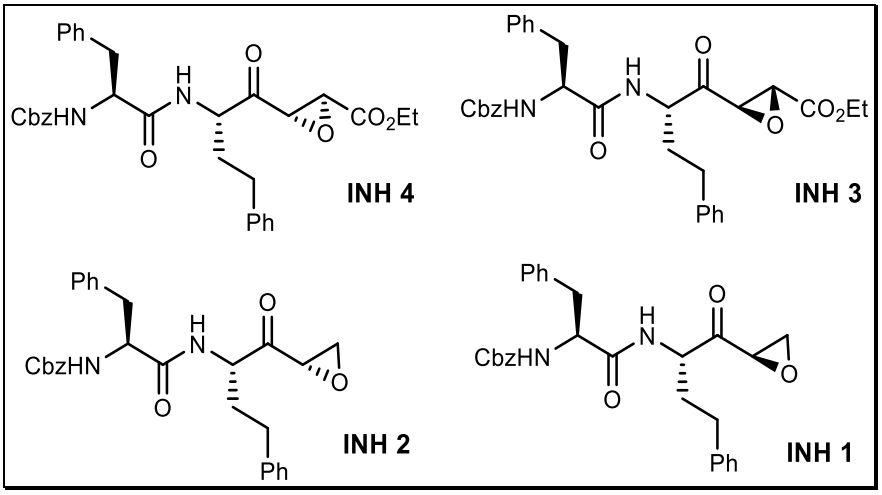



**Figure 14**. Dipeptidyl epoxy-compounds

**Table 1: Inhibition result $k_{inact}/K_i$ ($s^{-1}M^{-1}$)**

| Compound | cruzain | rhodesain | cathepsin B |
|---|---|---|---|
| **INH 1** | 128.200 | - | - |
| **INH 2** | 330.000 | - | - |
| **INH 3** | 82.900 | 92.090 | 120 |
| **INH 4** | 25.200 | 23.500 | 84.5 |
| **E-64c** | 70600 | - | - |

[88] Varughese K, Ahmed F, Carey P, Hasnain S, Huber C, Storer A. *Biochemistry* **1970**, 28, 1332–2
[89] Roush, W. R.; González, F. V.; Hanssell, E.; McKerrow, J. H. *Bioorg. Med. Chem. Lett.* **1998**, 8, 2809-2812
[90] González, F. V.; Izquierdo, J.; Rodríguez, S.; McKerrow, J. H.; Hanssell, E. *Bioorg. Med. Chem. Lett* **2007**, 6697-6600

The first peptidyl α,β-epoxyketones described in the literature are the natural microbial products epoxomicin and eponemycin (**figure 15**).[91,92]

Epoxomicin, an α,β-epoxyketone, is a natural microbial metabolite, which was first isolated from *Actinomyces* due to its in vivo antitumor activity against murine B16 melanoma tumors. Despite this potent activity, the mechanism of biological action has not been definitely elucidated.

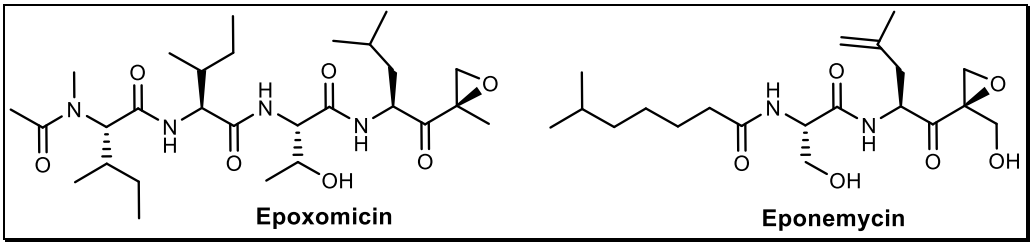



**Figure 15.** Epoxomicin and Eponemycin compounds

Spaltenstein and co-workers, in search of a selective proteasome inhibitor, were the first to report in 1996 enzyme inhibitors that inhibited the proteasome. The tripeptide α,β-epoxyketone Cbz-Ile-Ile-Phe-(2*R*)-EP inhibits the chymotrypsin-like activity of the 20S proteasome at low nanomolar concentrations ($IC_{50}$ = 5 nM).[93] Shortly after the discovery of this new class of compounds that inhibited the proteasome, the search began to identify other inhibitors of the proteasome that incorporated the α,β-epoxyketones pharmacophore. This

[91] Sugawara, K.; Hatori, M.; Nishiyama, Y.; Tomita, K.; Kamei, H.; Konishi, M.; Oki, T. *J. Antibiot.* **1990**, *43*, 8
[92] Hanada, M.; Sugawara, K.; Kaneta, K.; Toda, S.; Nishiyama, Y.; Tomita, K.; Yamamoto, H.; Konishi, M.; Oki, T. *J. Antibiot.* **1992**, *45*, 1746
[93] Spaltenstein, A.; Leban, J. J.; Huang, J. J.; Reinhardt, K. R.; Viveros, O. H.; Sigafoos, J.; Crouch, R. *Tetrahedron Lett.* **1996**, *37*, 1343-1346

inhibition results suggested that other α,β-epoxyketones, such as Epoxomicin and its derivatives, could target and inactivate the proteasome and could be used as molecular probes and therapeutic agents.

A mechanism has been recently suggested for the inhibition of subunit $\beta_5$ of proteasome by epoxomycin,[94] **figure 16**.

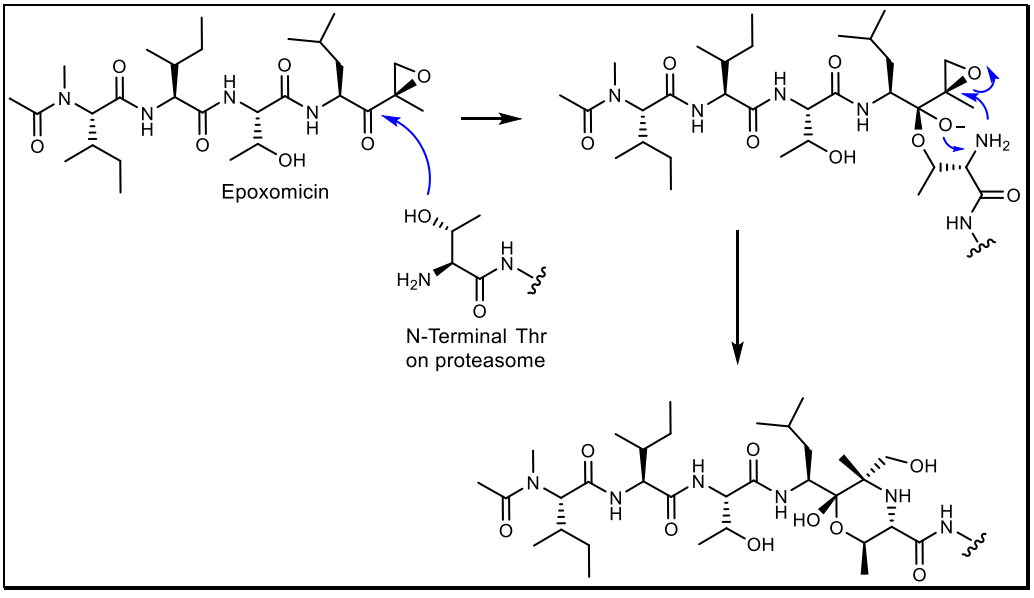



**Figure 16**. Inhibition mechanism of Epoxomycin

### 1.7.2. Halomethyl ketones

As an extension of previous work in serine protease inhibitors, these halo methyl ketones (HMK) demonstrated to be one of the most interesting inhibitors. Since Rasnik[95] and Shaw[96] developed fluoromethyl ketones (FMK), several new

[94] Groll, M.; Kim, K. B.; Kairies, N.; Huber, R.; Crews, C. M. *J. Am. Chem. Soc.* **2000**, 122, 1237– 1238
[95] Rasnick, D. *Anal. Biochem.* **1985**, *149*, 461-465

inhibitors emerged with different leaving groups like chloromethyl ketones (CMK, **figure 17**) which are irreversible inhibitors.

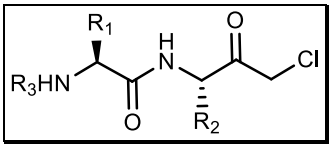



**Figure 17**. General structure of a CMK inhibitor

Peptidyl halomethyl ketones also inhibit a variety of cysteine proteases such as papain, cathepsins B, H, and L, calpains, and caspases.

### 1.7.3. Aziridines

Aziridines contain a reactive aziridine-2,3-dicarboxylic acid or aziridine-2-carboxylic acid moiety attached to a peptide or amino acid residue. Three types of aziridine inhibitors of cysteine proteases have been described[97] according to the moieties surrounding the aziridine group, illustrated in **figure 18**.

[96] Rauber, P.; Angliker, H.; Walker, B.; Shaw, E. *Biochem. J.* **1986**, *239*, 633
[97] Schirmeister, T. *J. Med. Chem.* **1999**, *42*, 560

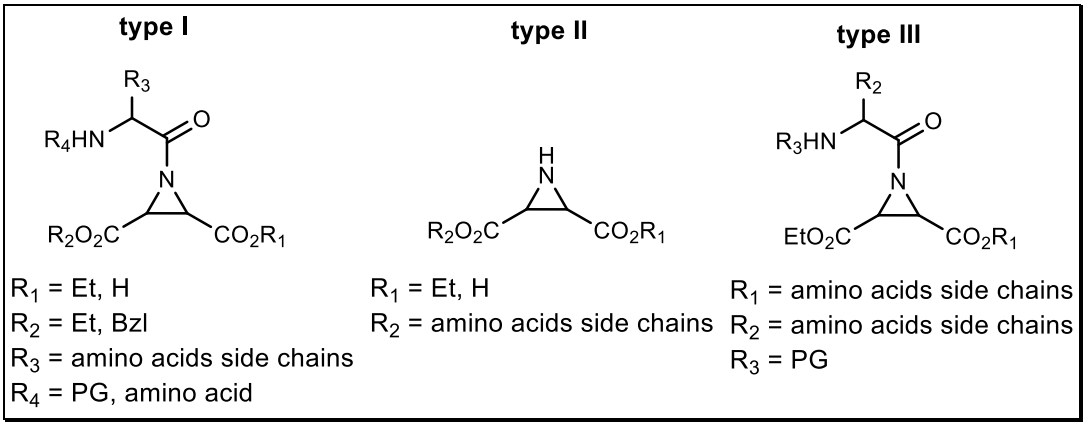



**Figure 18.** Aziridine inhibitors types

These compounds are irreversible inhibitors of the cysteine proteases papain, cathepsins B, L, and H. The proposed mechanism of inhibition of cysteine proteases by aziridines is similar to that proposed for epoxysuccinyl derivatives. However, it is known that aziridines inhibit most effectively at low pH values.[98] This increased inhibitory reactivity is due to the protonation of the aziridine nitrogen possibly by the active site histidine residue. The protonated aziridine can also interact with the oxyanion hole of cysteine proteases with energetically favorable hydrogen bonding.[99] Although both the aziridine and the epoxysuccinyl may have similar chemical reactivities, differences exist between the two classes of inhibitors.

Ready protonation of the nitrogen of type II aziridines is one difference between aziridines and their epoxysuccinyl analogues. A second difference is the hydrogenbonding abilities of the two classes of inhibitors. Aziridines are H-bond donors, whereas the epoxysuccinyl inhibitors are H-bond acceptors. These differences suggest that the two classes of inhibitors may have different binding

[98] Martichonok, V.; Plouffe, C.; Storer, A. C.; Menard, R.; Jones, J. B. *J. Med. Chem.* **1995**, *38*, 3078
[99] Schirmeister, T. *Biopolymers* **1999**, *51*, 87

modes and possibly variable interactions with cysteine proteases. Last, unlike most epoxysuccinyl inhibitors, the *R,R*-configuration of the aziridine ring is preferred for inhibition in both types II and III aziridine inhibitors, whereas the type I aziridine inhibitors with the *S,S*-configuration are better inhibitors.

A natural product, Miraziridine (**figure 19**) is an aziridinesuccinyl derivative isolated from the marine sponge *Theonella mirabilis.*[100]The $IC_{50}$ value for cathepsin B is 1.4 μg/mL and was the first reported vinyl arginine residue

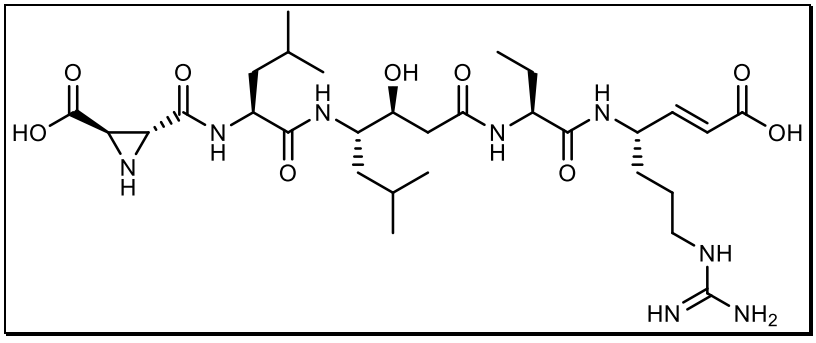



**Figure 19**. Structure of Miraziridine

### 1.7.4. Michael acceptors inhibitors

One of the first Michael acceptors described in the literature is the fumarate derivative of E-64c, **figure 20**. This inhibitor contains an α,β-unsaturated carboxyl moiety and was found to be an irreversible inhibitor of cathepsin B ($k_{app}$ = 625 $M^{-1}$ $s^{-1}$), cathepsin H ($k_{app}$ = 11 $M^{-1}$ $s^{-1}$), and cathepsin L ($k_{app}$ = 2272 $M^{-1}$ $s^{-1}$).[101]

[100] Nakao, Y.; Fujita, M.; Warabi, K.; Matsunaga, S.; Fusetani, N. *J. Am. Chem. Soc.* **2000**, *122*, 10462
[101] Barrett, A. J.; Kembhavi, A. A.; Brown, M. A.; Kirschke, H.; Knight, C. G.; Tamai, M.; Hanada, K. *Biochem. J.* **1982**, *201*, 189-198

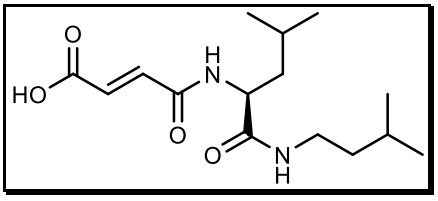


**Figure 20**. E-64c derivative

Hanzlik and co-workers were the first group to design a series of amino acid derived Michael acceptor inhibitors for cysteine proteases. They showed that various vinyl sulfone and α,β-unsaturated carbonyl derivatives of phenylalanine are inhibitors of papain and dipeptidyl peptidase I (DPPI).[102,103] Hanzlik and co-workers then incorporated a range of Michael acceptor electrophiles into dipeptide analogues and studied their effects on papain.[104]The most reactive derivatives with papain were an unsaturated ester and a simple vinyl sulfone as **figure 21** shows.

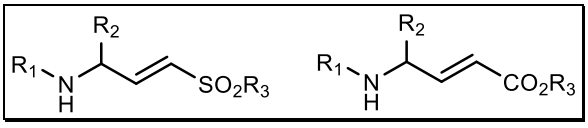


**Figure 21**. Vinyl sulfone and α,β-unsaturated carbonyl compounds

These vinyl sulfone cysteine protease inhibitors were later developed into a class of inhibitors for a variety of other cysteine proteases by Palmer and co-workers. They designed potent inhibitors against disease-associated cysteine proteases, such as cathepsins B, L, S, and K (also called cathepsin O2), calpains, and cruzain.[105] This class of irreversible inhibitors, which contain a double bond

[102] Hanzlik, R. P.; Thompson, S. A. *J. Med. Chem.* **1984**, *27*, 711-712
[103] Thompson, S. A.; Andrews, P. R.; Hanzlik, R. P. *J. Med. Chem.* **1986**, *29*, 104-111
[104] Liu, S.; Hanzlik, R. P. *J. Med. Chem.* **1992**, *35*, 1067-1075
[105] Palmer, J. T.; Rasnick, D.; Klaus, J. L.; Brömme, D. *J. Med. Chem.* **1995**, *38*, 3193-3196

activated by an electron-withdrawing sulfone functional group, was subsequently shown also to inhibit the rhinovirus 3C protease and cathepsin V.[106,107]

Peptidyl vinyl sulfones and other Michael acceptors are stable, unreactive toward nucleophiles, and need the catalytic machinery of the cysteine proteases for activation. Vinyl sulfones are less reactive toward nucleophiles than vinyl ketones or vinyl esters (summarized in **table 2**), which is an advantage for *in vivo* studies. In addition, vinyl sulfones can be manipulated on both the P and P´site of the molecule, allowing for greater selectivity and reactivity toward target enzymes (illustrated in **figure 22**). Thus, most of the Michael acceptors, which have been studied, are vinyl sulfones.

**Table 2: Inhibition of Papain by Ac-Phe-NH-$CH_2$-CH=CH-R with different EWG**

| R | $k_2/K_i$ ($M^{-1}$ $s^{-1}$) |
|---|---|
| $COOCH_3$ | 26.1 |
| $SO_2CH_3$ | 18.7 |
| COOH | 5.0 |
| CN | 1.7 |
| $CONH_2$ | 1.1 |
| $PhNO_2$ | - |

[106] Dragovich, P. S.; Webber, S. E.; Babine, R. E.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Lee, C. A.; Reich, S. H.; Prins, T. J.; Marakovits, J. T.; Littlefield, E. S.; Zhou, R.; Tikhe, J.; Ford, C. E.; Wallace, M. B.; Meador, J. W., 3rd; Ferre, R. A.; Brown, E. L.; Binford, S. L.; Harr, J. E.; DeLisle, D. M.; Worland, S. T. *J. Med. Chem.* **1998**, *41*, 2806-2818

[107] Somoza, J. R.; Zhan, H.; Bowman, K. K.; Yu, L.; Mortara, K. D.; Palmer, J. T.; Clark, J. M.; McGrath, M. E. *Biochemistry* **2000**, *39*, 12543-12551

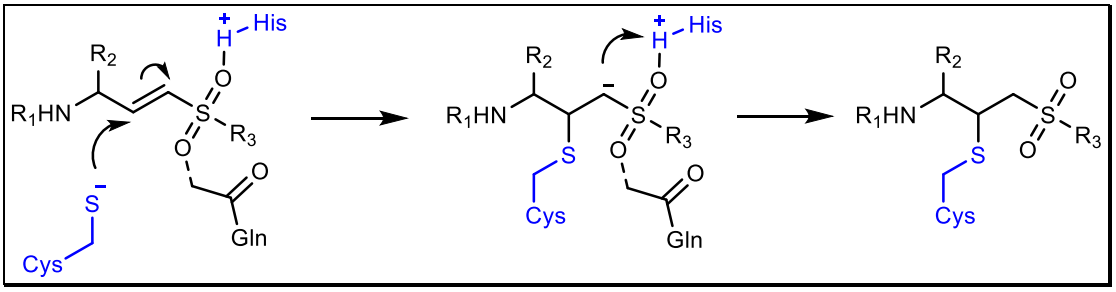



**Figure 22**. Proposed vinyl sulfone inhibition mechanism

Roush and co-workers have carried out extensive structure activity relationship (SAR) studies with vinyl sulfone inhibitors of cruzain to develop new therapies for Chagas' disease. Cruzain is the major cysteine protease of *T. cruzi*, the causative agent of Chagas' disease. Initially, Roush et al. expanded on Palmer's work with Mu-Phe-Hph-VS-Ph ($k_{inact}/K_i$ = 203000 $M^{-1}$ $s^{-1}$) and made a number of structural variants to design potent and selective inhibitors of cruzain.[108] The crystal structure of cathepsin K with the vinyl sulfone APC3328 revealed that the phenyl residue of the vinyl sulfone unit did not make optimal interactions with prime site residues represented in **figure 23**.[109]

[108] Roush, W. R.; Gwaltney, S. L., II; Cheng, J.; Scheidt, K. A.; McKerrow, J. H.; Hansell, E. *J. Am. Chem. Soc.* **1998**, *120*, 10994-10995

[109] McGrath, M. E.; Klaus, J. L.; Barnes, M. G.; Brömme, D. *Nat. Struct. Biol.* **1997**, *4*, 105-109

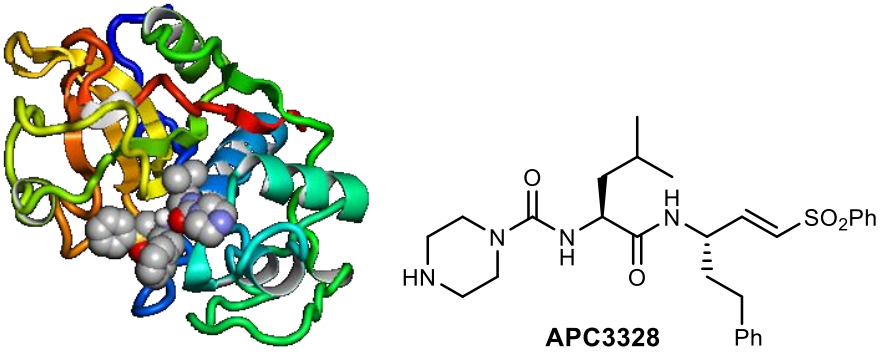



**Figure 23**. Complex cathepsin K with APC3328

Roush et al. synthesized vinyl sulfonamides and vinyl sulfonate esters to enhance the prime site interactions. These inhibitors have the structure Cbz-Phe-Hph-VS-R, where R = Ph, $CH_2Ph$, $CH_2CH_2Ph$, NHPh, and OPh, and were found to be time-dependent inhibitors of cruzain, papain, and cathepsin B. Vinyl sulfonamides are designated peptidyl-VS-NH-R, where R = alkyl or aryl, whereas vinyl sulfonate esters are designated peptidyl-VS-O-R, where R = aryl. Roush discovered the most potent cruzain inhibitor reported to date shown in **figure 24**, Cbz-Phe-Hph-VS-OPh ($k_{assoc}$ = 16800000 $M^{-1}$ $s^{-1}$), which has the preferred one-atom oxygen spacer between the sulfonyl unit and phenyl ring.[110]

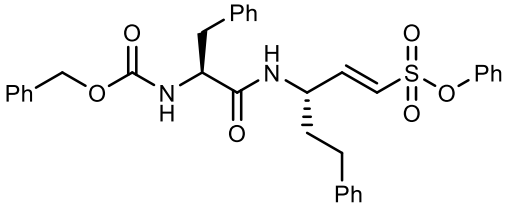



**Figure 24**. Roush' vinyl sulfonate inhibitor for cruzain

[110] Roush, W. R.; Cheng, J.; Knapp-Reed, B.; Alvarez-Hernandez, A.; McKerrow, J. H.; Hansell, E.; Engel, J. C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2759-2762

## 1.8. Activity based probe (ABP) and affinity based protein profiling (ABPP)

Recently one of the most studied fields is the research of proteins especially in the active mode. Proteomics focus the attention in the study of these proteins and their interactions, functions… and finally the understanding of the function of one protein in a physiological process. ABP provides a direct chemical probe of the activity and mechanism in the active site of an enzyme only for catalytically-activated species.

A typical ABP are formed by 3 essential elements: First one is the reactive part, normally consist in a warhead that can form a covalent union with the target protein and an amino sequence to increase the affinity and specificity in the active site. Second one is the linker, which provides the necessary space between the reactive part and the tag, element used for identification or location. The last part is the tag allowing an easy and quick detection and simple purification. Normally an affinity tag (biotin) is used for isolation proteins and fluorescent tag (BODIPY) for quantification and monitoring, illustrated in **figure 25**.


| Warhead | Linker | Tag |
|---|---|---|
| Epoxides, phophonates, sulfonates, vinyl… | Alkyl, peptide, glycol… | Biotin, Bodipy, radioactive… |

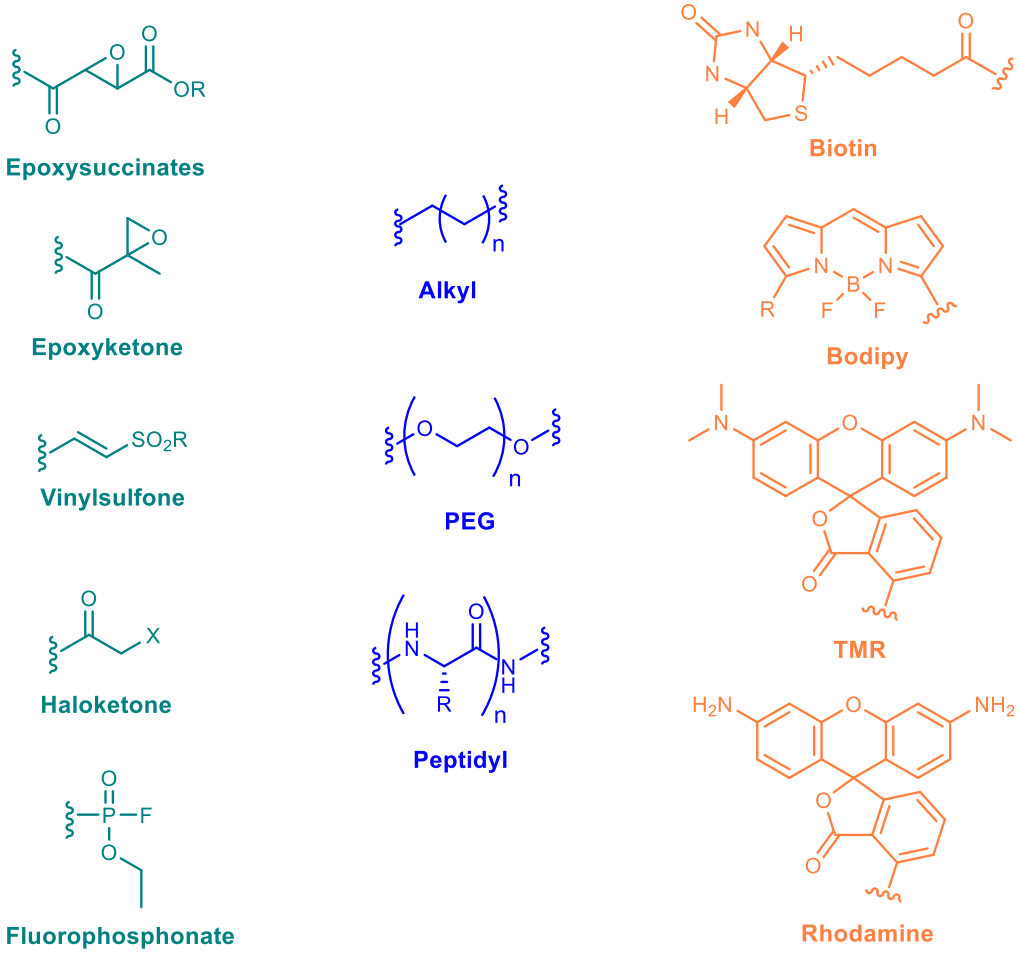



**Figure 25**. General ABP structures

One of the most important tools in the proteomic studies is ABPP, an analytical technique which is useful in complex proteins. The identification of proteomes (normal and diseased state, in separately assays) with ABPP as biomarkers is a powerful application. It can be separated and identified the active enzymes after this ABPP incubation application by SDS-PAGE. A final identification using a mass spectrometry analysis allows identifying the interest enzymes. It is summarized in **figure 26**, the ABPP process of the diseased state and normal state (done in separate assays) in a same picture.

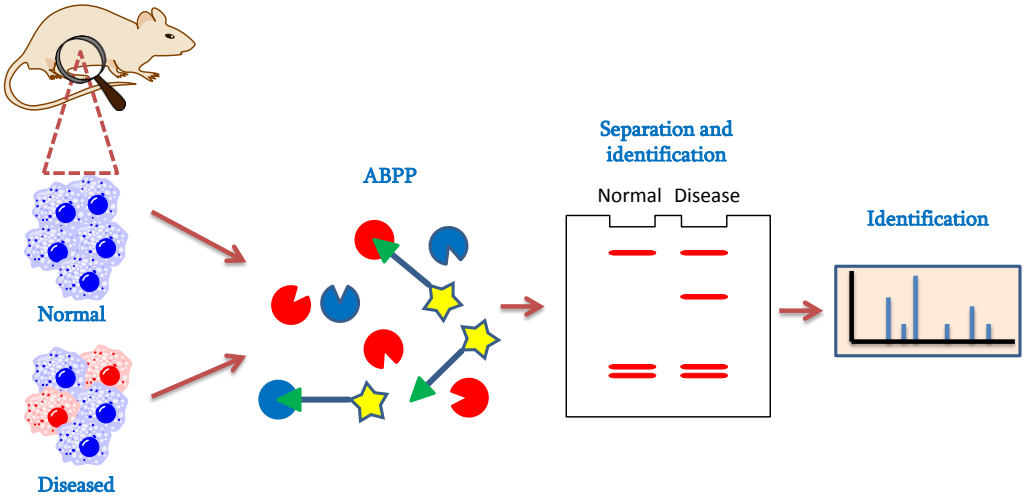



**Figure 26**. ABPP application process

This same methodology can be applied in cancer field to *in vivo* profiling cathepsins activity. The monitoring is due to BODIPY presence using ABPP in mice. A simple comparison of located signal in normal islets, in tumors and with the corresponding inhibitor can illustrated the decrement in the number of the tumor islets (**figure 27**).[111]

[111] Joyce, J. A., Baruch, A., Chehade, K., Meyer-Morse, N., Giraudo, E., Tsai, F. Y., Greenbaum, D. C., Hager, J. H., Bogyo, M., Hanahan, D. *Cancer Cell*, **2004**, *5*, 443-453

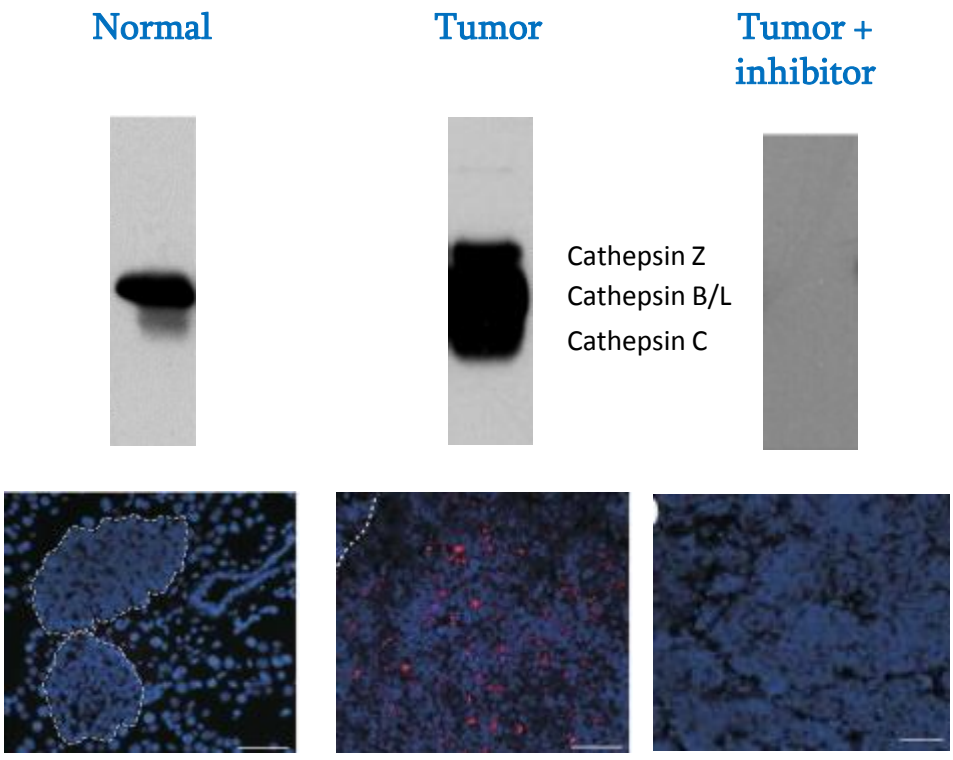



**Figure 27**. ABPP application on tumor cells

Finally, according to the methodology used during this PhD, ABPP was used as a strong tool to carry out competitive assays between different small molecules as inhibitor to check as a routine procedure the inhibition capacity compared with a well-known inhibitor.

Treatment of different new inhibitors with proteasome and subsequent ABPP protocol and SDS-PAGE, can contribute to rewarding information about the new inhibitors. New inhibitors should be easily evaluated (separately way), the more potent inhibitor the more decrease in the intensity in the gel analysis seen in **figure 28**.

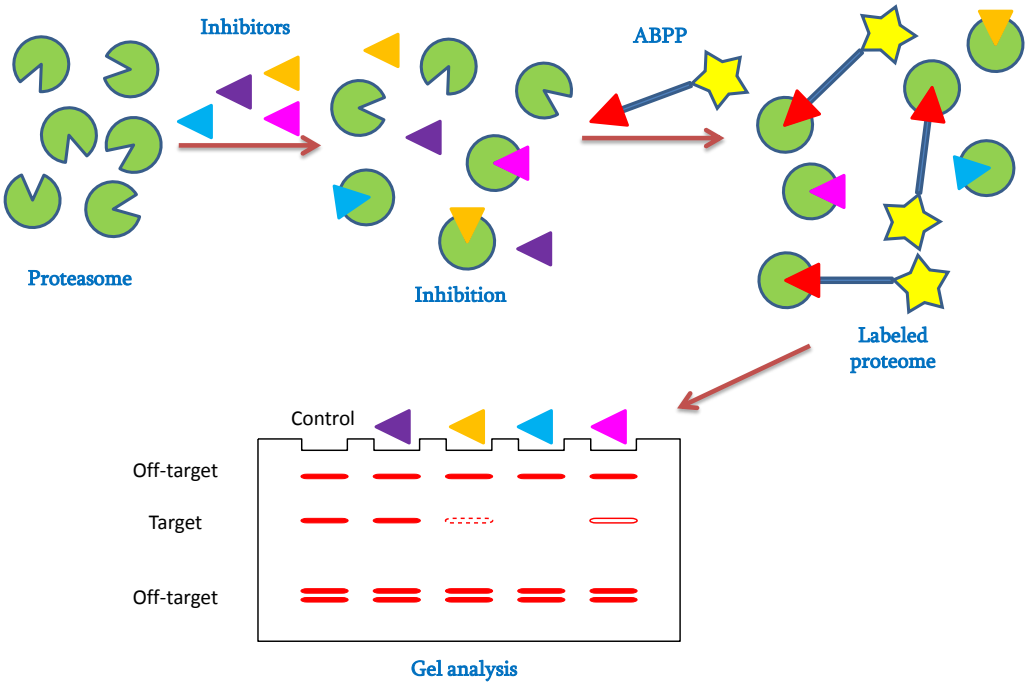



**Figure 28**. ABPP competitive assay

It also works with a new inhibitor confirming the low level of concentration of inhibitor not detecting the enzyme activity. This concentration (**figure 29**) could be compared with other literature inhibitors and extract interesting conclusions.

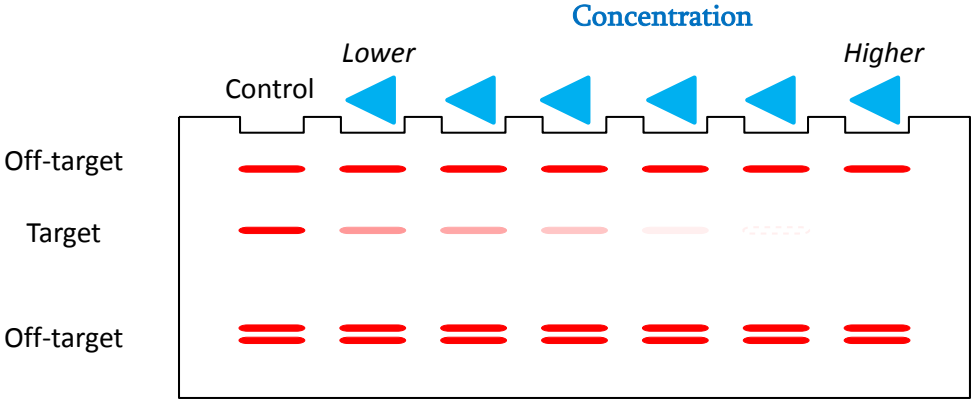



**Figure 29**. ABPP assay for minimum concentration

# 2. Synthesis and results

The aim of this work is to develop new families of inhibitors against parasitic cysteine proteases and proteasome enzyme. Initially, in our group only developed cysteine proteases inhibitors for parasitic diseases, but since my research stay in the Prof. Dr. Overkleeft, theses new inhibitors families were tested against proteasome too.

New warheads could be designed and synthetized based on previously described inhibitors, then taking in account best amino acid sequence and making a new warhead a wide range of new inhibitors could be made. The general structure is represented in **figure 30**.

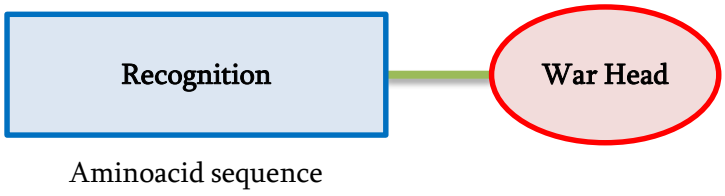



**Figure 30**. General structure of designed new compounds

## 2.1. New nitro compounds as inhibitors

The synthesis of this family of new inhibitors was based on the structure of already known dipeptidyl vinyl sulfones. It was presumed that the substitution of a sulfone by a nitro group could afford a new type of reversible inhibitors. Based on the mechanism since nitroalkanes are more acidics than imidazole (pKa in DMSO for nitroalkane= 17.2; sulfone= 29; imidazole= 23) the intermediate anion resulting from the Michael addition of enzyme thiolate to the double bond of nitroalkene would not be protonated by imidazolium Histidine. Reversely, sulfones are less acidic than imidazoles and vinyl sulfones inhibitors are irreversible inhibitors of cysteine proteases.

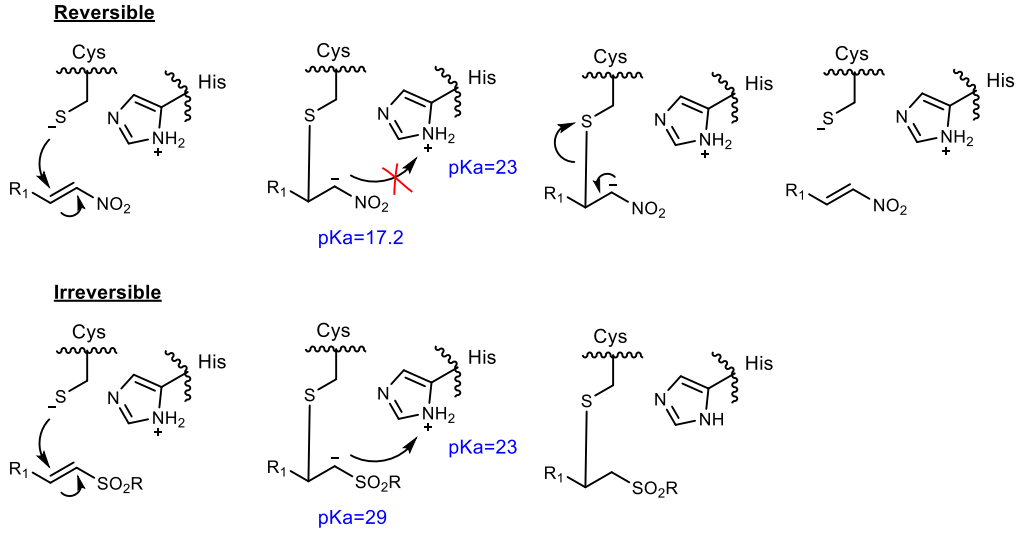



**Figure 31**. Comparison of nitro and sulfones inhibitors

Also the synthetic versatility of nitroalkenes could lead to other type of inhibitors through epoxidation, cyclopropanation… (**figure 32**).

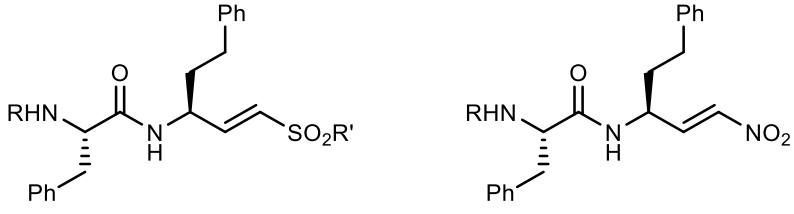
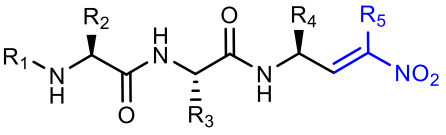
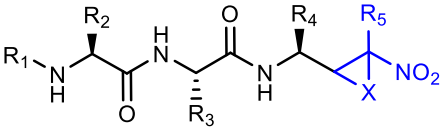


**Figure 32**. Structure comparison of nitro and sulfone compounds

This family of new inhibitors based on the presence of a nitro group is showed below in **figure 33** and big ranges of transformation are based on other previously described inhibitors such as epoxides, aziridines or ciclopropanes.



$R_1$ = PG
$R_2$,$R_3$,$R_4$ = amino acids side chains
$R_5$ = H or Alkyl



$R_1$ =PG
$R_2$,$R_3$,$R_4$ = amino acids side chains
$R_5$ = H or Alkyl
X = O, NH, $CH_2$

**Figure 33**. Structure designed for new nitro compounds

The retrosynthetic analysis for this nitrocompounds is represented in the next **figure 34** affording the nitroalkenes and other modified nitrocyclopropanes.

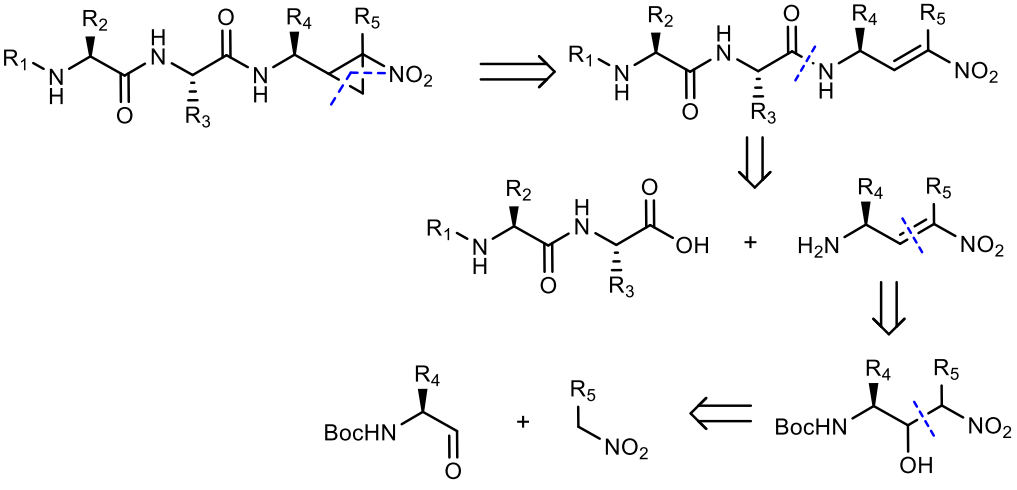


**Figure 34**. Retrosynthetic analysis for new nitro compounds

### 2.1.1. Dipeptidyl/tripeptidyl nitroalkenes

The work started on the preparation of the nitroalkene moiety as the most similar with vinyl sulfones. According to previously retrosynthetic analysis, N-protected amino aldehydes were obtained from commercially available amino acid with Weinreb methodology or by using diisobutyl aluminum hydride as illustrated in **figure 35**.

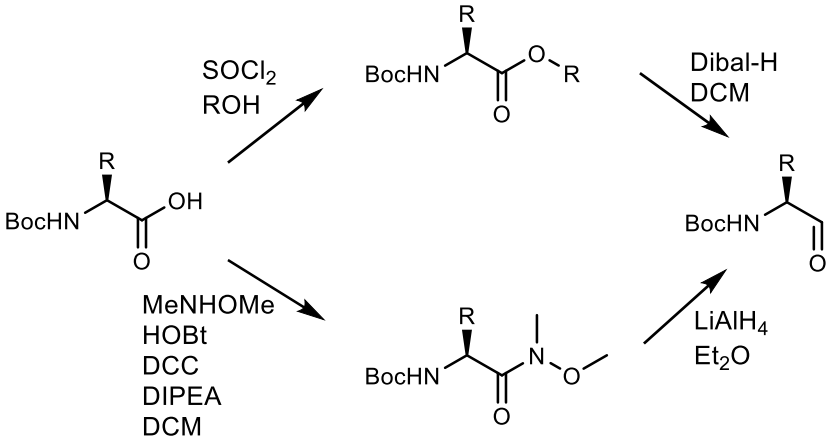
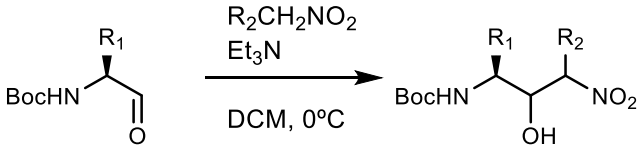



**Figure 35**. Preparation of amino aldehyde precursor

Aldehydes were submitted to a nitroaldol reaction (**scheme 4**) using an excess of nitromethane or nitroethane (usually 6 equivalents) in dichloromethane and a catalytic amount of triethylamine. Nitroaldols were obtained in excellent chemical yields as inseparable mixtures of two diastereoisomers (around 50/50

ratio) in the case of nitromethane or four diastereoisomers (about 25% de each diastereoisomer) in the case of nitroethane, summarized in **table 3**.



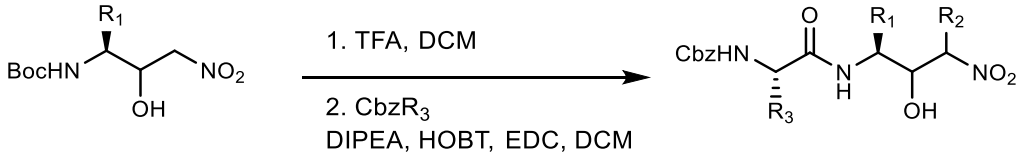

**Scheme 4**. Nitroaldol reaction

**Table 3. Nitroaldol reaction results**

| Entry | $R_1$ | $R_2$ | Yield (%)[a] |
|---|---|---|---|
| 1 | $CH_3$ | H | 95 |
| 2 | *i*-Butyl | H | 90 |
| 3 | Bn | H | 85 |
| 4 | $(CH_2)_2Ph$ | H | 93 |
| 5 | $CH_3$ | Me | 88 |
| 6 | *i*-Butyl | Me | 77 |
| 7 | Bn | Me | 86 |
| 8 | $(CH_2)_2Ph$ | Me | 87 |

[a] Yield for the diastereoisomeric mixture

The mixture of nitroaldols was submitted to amino deprotection conditions with trifluoroacetic acid in dichloromethane and subsequent amino coupling with the desired amino acid affording dipeptidyl nitro aldols as diastereoisomeric mixture in good chemical yields as can be seen in **scheme 5**,**table 4**.

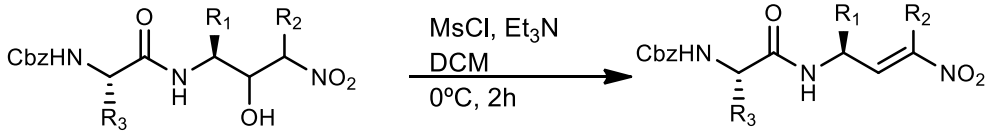



**Scheme 5**. Deprotection and coupling reactions

**Table 4. Deprotection and coupling results**

| Entry | $R_1$ | $R_2$ | $R_3$ | Yield (%) |
|---|---|---|---|---|
| 1 | $CH_3$ | H | Bn | 78 |
| 2 | $CH_3$ | H | *i*-Butyl | 86 |
| 3 | *i*-Butyl | H | Bn | 85 |
| 4 | *i*-Butyl | H | *i*-Butyl | 69 |
| 5 | Bn | H | Bn | 74 |
| 6 | Bn | H | *i*-Butyl | 71 |
| 7 | $(CH_2)_2Ph$ | H | Bn | 86 |
| 8 | $(CH_2)_2Ph$ | H | *i*-Butyl | 81 |
| 9 | $CH_3$ | Me | Bn | 71 |
| 10 | $CH_3$ | Me | *i*-Butyl | 73 |

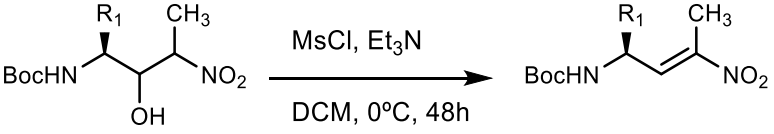
Finally these nitroaldols mixtures were treated with mesyl chloride in dichloromethane with triethylamine as a base affording the desired elimination product (**scheme 6**) in good yields as a single isomer for $R_2$= H and as an E/Z mixture of compounds for $R_2$= Me (**table 5**).




**Scheme 6**. Elimination reaction

| Table 5. Elimination results | | | | | |
|---|---|---|---|---|---|
| **Product** | **Entry** | **$R_1$** | **$R_2$** | **$R_3$** | **Yield (%)** |
| **4.1** | 1 | $CH_3$ | H | Bn | 93 |
| **4.2** | 2 | $CH_3$ | H | *i*-Butyl | 88 |
| **4.3** | 3 | *i*-Butyl | H | Bn | 81 |
| **4.4** | 4 | *i*-Butyl | H | *i*-Butyl | 74 |
| **4.5** | 5 | Bn | H | Bn | 89 |
| **4.6** | 6 | Bn | H | *i*-Butyl | 75 |
| **4.7** | 7 | $(CH_2)_2Ph$ | H | Bn | 92 |
| **4.8** | 8 | $(CH_2)_2Ph$ | H | *i*-Butyl | 82 |
| **4.9** | 9 | $CH_3$ | Me | Bn | 71 |
| **4.10** | 10 | $CH_3$ | Me | *i*-Butyl | 64 |

For the preparation of α-methylated nitroalkenes (**scheme 7**), same reactions sequences were employed but elimination step needed longer time and more equivalents for reaction go to completion.




**Scheme 7**. Elimination reaction of α-methylated nitroalkenes

*In vitro* testing against cysteine proteases of prepared dipeptidyl nitroalkenes represented in **figure36** are currently carried out.

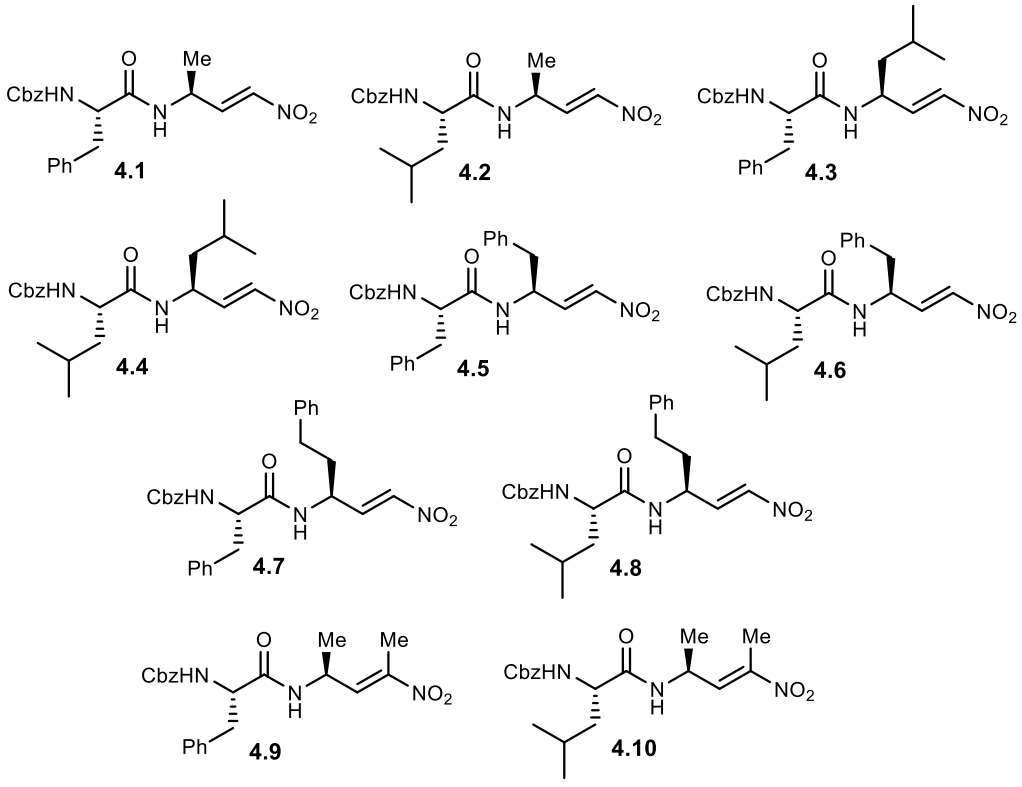



**Figure 36**. Dipeptidyl nitroalkenes prepared

Dipeptidyl nitroalkenes have been recently patented (**EP14382307**) and we expected be published in a medicinal chemistry journal in the next few months.

On the other hand the structural analogy of nitroalkenes with vinyl sulfones can be seen in a docking study. It was compared the known K11777 vinyl sulfone (orange) with one nitroalkene **4.1**(green) in the active site. It was illustrated in **figure 37**.

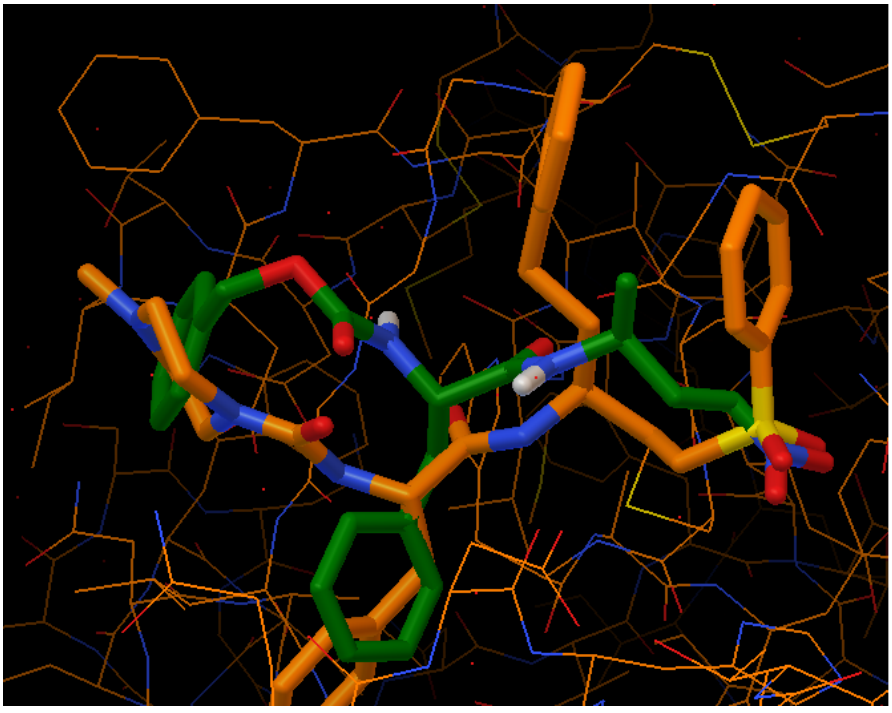
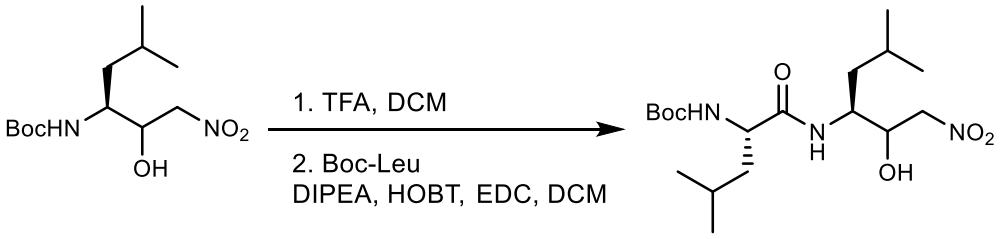


**Figure 37**. Docking study of nitroalkenes **4.1**

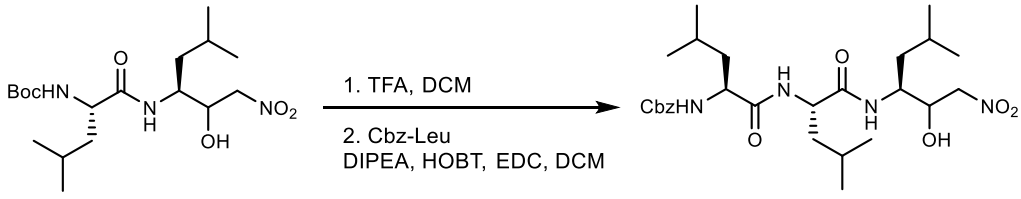
As it can be seen, the orientation of two amino sequences are the same, maximizing the interactions in the pockets and reducing the energy in both cases. In the warhead part, the double bond is in the same position and the orientation. Furthermore, two oxygens of the nitro group in exactly the same position as the two oxygens of the sulfone.

A tripeptidyl nitroalkene inhibitor having three L-leucine aminoacids was also prepared. The preparation of this compound started from Boc-nitroaldol derived from leucine which was deprotected with trifluoroacetic acid in dichloromethane and coupled with Boc-Leucine under standard conditions to give the desired dipeptidylnitroaldol, (yield = 79%) **scheme 8**.



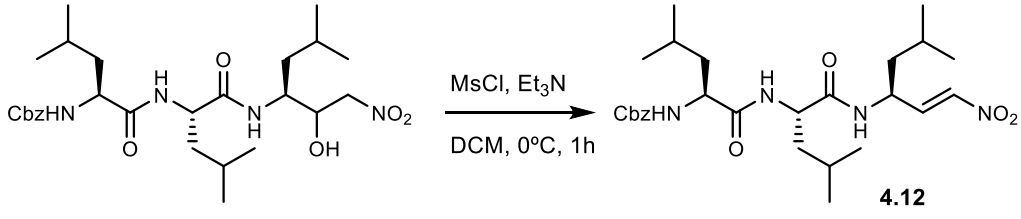

**Scheme 8**. Deprotection and coupling reactions

Same sequence was applied to resulting modified dipeptide but using Cbz-Leucine giving rise to corresponding tripeptidyl nitroaldol (yield = 69%), **scheme 9**.




**Scheme 9**. Deprotection and coupling reactions

Finally, the nitroaldol was submitted to elimination, **scheme 10**, by activation of alcohol group into mesylate which eliminates to afford the desired tripeptidyl nitroalkene **4.12** (yield = 67%).



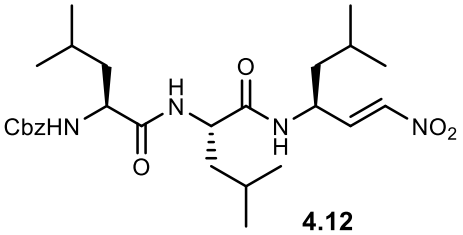

**Scheme 10**. Elimination reaction

### 2.1.2. Inhibition testing

*In vitro* testing against cysteine proteases are currently carried out.

Biological test in Hela cell lysate

The standard protocol was carried out with 1h of incubation, 1h labelling of residual activity with LWA-300 (green bodipy-epoxomicin) and resolve on 12.5 SDS-PAGE. The inhibition results are illustrated for inhibitor **4.12** in **figure 38**.




**4.12**

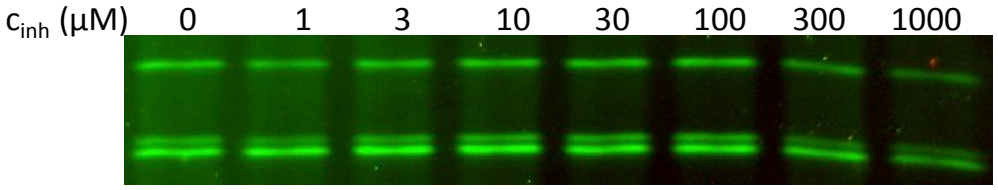



**Figure 38**. SDS-PAGE for inhibitor **4.12**

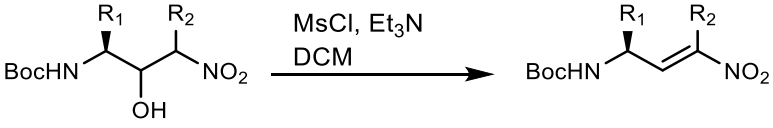
As it can be seen in the figure, the inhibitor needs high concentrations to observe the inhibition effect on the intensity of the signal in all the targets. This concentration is too much high to consider compound **4.12** as a good inhibitor. On

the other hand, the same intensity decrease in all targets suggests that inhibitor is not specific for any target.

These results might be due to the expected reversibility of nitroakenes. Proteasome inhibitors needs to be irreversible as is the case for epoxomicin to achieve good inhibition results.

### 2.1.3. Dipeptidyl nitro cyclopropanes

Nitroaldol mixtures were submitted to elimination conditions, **scheme 11**, with mesylchloride in dichloromethane with triethylamine to obtain the corresponding nitroalkenes in excellent yields as it can be seen in **table 6**.

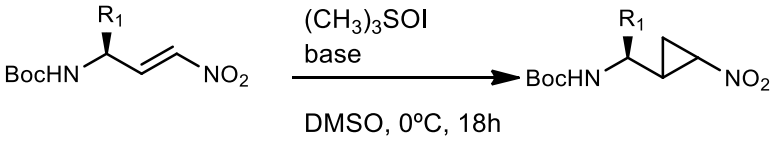



**Scheme 11**. Elimination reaction

**Table 6. Elimination results**

| Compound | Entry | $R_1$ | $R_2$ | Yield (%) |
|---|---|---|---|---|
| **4.13** | 1 | $CH_3$ | H | 95 |
| **4.14** | 2 | *i*-Butyl | H | 90 |
| **4.15** | 3 | Bn | H | 85 |
| **4.16** | 4 | $(CH_2)_2Ph$ | H | 93 |
| **4.17** | 5 | $CH_3$ | Me | 88 |

The obtained nitroalkenes were treated with sulfur ylide derived from trimethyl sulfoxonium iodine treated with sodium hydride or potassium *terc*-butoxide in dimethylsulfoxide affording a 50/50 mixture of *trans* cyclopropanes as shown in **table 7** and **scheme 12**. Simmons-Smith cyclopropanation conditions using diethyl zinc and diiodomethane in dichloromethane were not successful.

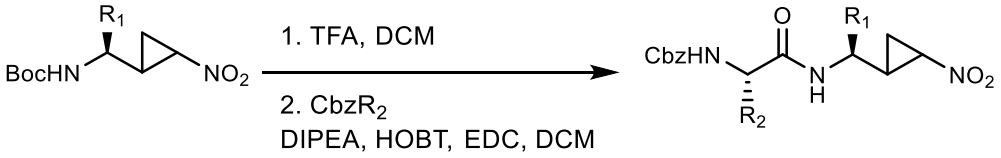



**Scheme 12**. Cyclopropanation reaction

**Table 7. Cyclopropanation assay**

| Entry | $R_1$ | Base | Yield (%) |
|---|---|---|---|
| 1 | $CH_3$ | NaH | 27.7 |
| 2 | *i*-Butyl | *t*-BuOK | 53.6 |
| 3 | Bn | *t*-BuOK | 34.8 |
| 4 | $(CH_2)_2Ph$ | *t*-BuOK | 37.2 |

After deprotecting Boc group with trifluoroacetic acid in dichloromethane, the resulting amine was coupled, **scheme 13**, with the desired Cbz-aminoacid with common coupling reagents (HOBT, EDC and DIPEA) in dichloromethane to form the dipeptidyl structure summarized in **table 8**.




**Scheme 13**. Deprotection and coupling reactions

**Table 8. Coupling results**

| Compound | Entry | $R_1$ | $R_2$ | Yield (%) |
|---|---|---|---|---|
| **4.18** | 1 | $CH_3$ | Bn | 74 |
| **4.19** | 3 | *i*-Butyl | Bn | 81 |
| **4.20** | 5 | Bn | Bn | 71 |
| **4.21** | 7 | $(CH_2)_2Ph$ | Bn | 79 |

*In vitro* testing against cysteine proteases of prepared dipeptidyl nitrocyclopropanes are currently carried out and it will be publish.

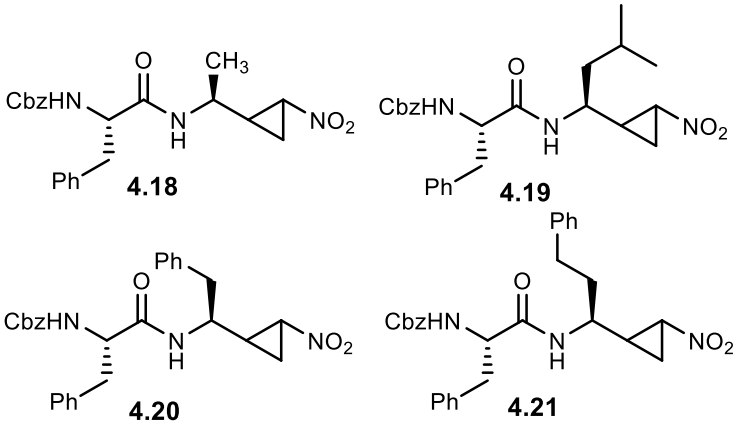
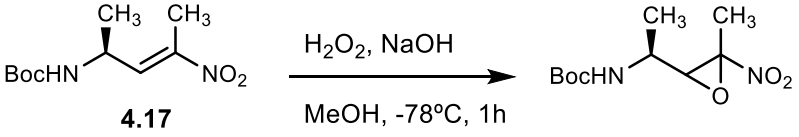



**Figure 39**. Dipeptidyl nitrocyclopropanes prepared

### 2.1.4. Nitroepoxides

Once dipeptidyl nitroalkenes were prepared, then we planned about the synthesis dipeptidyl nitroepoxides starting from already obtained nitroalkenes. Some epoxidation conditions (*n*-BuLi/*t*-BOOH, *m*-CPBA, $H_2O_2$/NaOH) were unsuccessful but when α-substituted nitroalkene **4.17** was treated with hydrogen peroxide and sodium hydroxide in methanol at -78 °C for 1h, a 6/4 mixture of isomeric epoxides was obtained, represented in **scheme 14**.

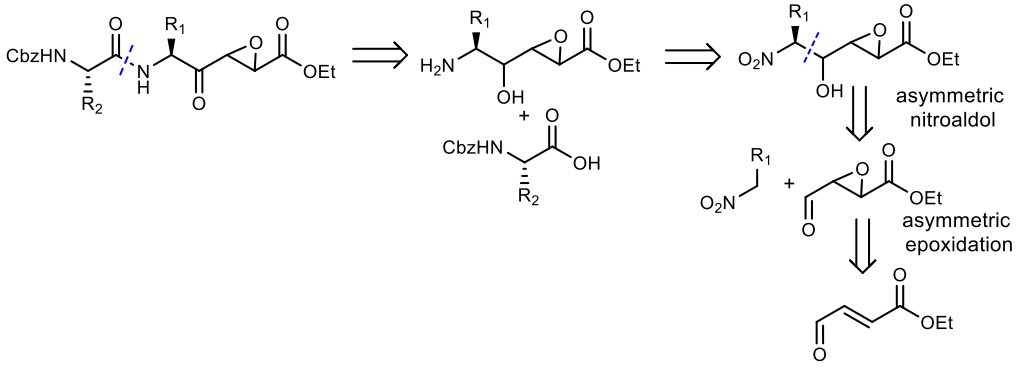



**Scheme 14**. Epoxidation reaction

Unfortunately resulting epoxides could not be purified by standard chromatography. The exploitation of these nitroepoxides is currently undergoing in our research group.

## 2.2. Amidation trough carbamates

As previously mentioned, dipeptidyl epoxiesters are potent cysteine proteases inhibitors synthesized in our group.[112] In order to prepare several grams of these compounds for *in vivo* testing, an improvement of previous synthetic route was required. The new route considered an asymmetric nitroaldol reaction between epoxialdehyde and nitroalkane as described in the **scheme 15.**

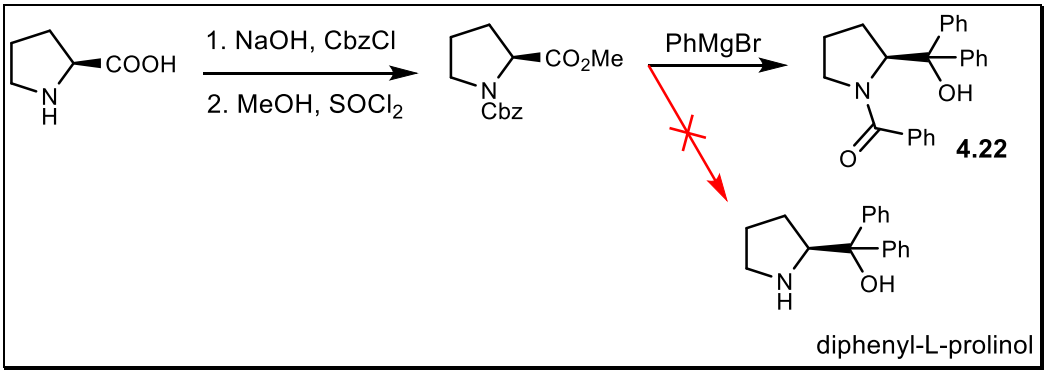



**Scheme 15**. New synthesis for dipeptidyl epoxyesteres

[112] González, F. V.; Izquierdo, J.; Rodriguez, S.; McKerrow, J. H.; Hansell, E. *Bioorg. Med. Chem.* Lett. **2006**, 17, 6697–6700

In order to prepare required epoxyaldehyde in an asymmetric fashion, our attention was focused on the asymmetric epoxidation of ethyl fumaraldehyde as previously reported.[113]

For the preparation of diphenyl-L-prolinol, Corey conditions were followed.[114] L-proline was N-protected as benzyl carbamate and then esterified as a methyl ester. Then resulting ester was treated with phenyl magnesium bromide, but unexpectedly prolinol **4.22** was obtained (**scheme 16**).




**Scheme 16**. Synthesis of diphenyl-L-prolinol

This result evoked us to study the reactivity of these carbamates due to carbamates and amides are widely used as protecting groups for amines.[115] Some reports described the conversion of carbamates to amides,[116,117,118] but a more convenient and general methods to accomplish this transformation was needed.

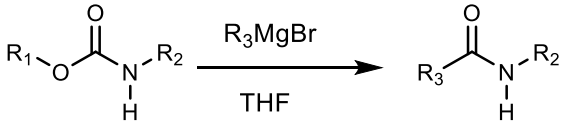
---

[113] Sundén, H.; Ibrahem, I.; Córdova, A.; *Tetrahedron Lett.* **2006**, *47*, 99-103
[114] Corey, E. J.; Shibata, S.; Bakshi, R. K. *J. Org. Chem.* **1988**, *53*, 2861-2863
[115] Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 4th ed.; Wiley: New York, **2006**
[116] Li, W.-R.; Yo, Y.-C.; Lin, Y.-S. *Tetrahedron* **2000**, *56*, 8867–8875
[117] White, J.D.; Blakemore, P. R.; Milicevic, S.; Choudhry, S. C.; Cupano, J.; Serico, L. *Org. Lett.* **2001**, *4*, 1803–1806
[118] El Kaim, L.; Grimaud, L.; Lee, A.; Perroux, Y.; Tirla, C. *Org. Lett.* **2004**, *6*, 381–383

### 2.2.1. Results and discussion

The idea was to set up a general and convenient procedure to transform carbamates of primary amines into a wide range of amides by treatment with available Grignard reagents.

Several amines (benzylamine, allylamine and 3-phenylpropylamine) were protected as carbamates (methyl, benzyl and *terc*-butyl) trough reaction with the corresponding alkoxy carbonyl chloride in the presence of sodium hydroxide.[3] The resulting carbamate was treated with a Grignard reagent for 24h in tetrahydrofuran giving in all cases amides, **scheme 17**, in high chemical yields as indicated in **table 9**.



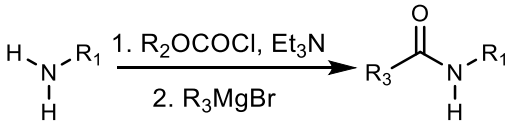

**Scheme 17**: General protected amines reaction

Table 9: Amides preparation

| Entry | $R_1$ | $R_2$ | Grignard ($R_3MgX$) | Yield (%) |
|---|---|---|---|---|
| 1 | Bn | Bn | MeMgBr | 66 |
| 2 | Bn | Bn | EtMgBr | 70 |
| 3 | Bn | Bn | PhMgBr | 73 |
| 4 | Bn | Bn | *t*-BuMgCl | 74 |
| 5 | Bn | $Ph(CH_2)_3$ | MeMgBr | 77 |
| 6 | Bn | $Ph(CH_2)_3$ | EtMgBr | 72 |
| 7 | Bn | $Ph(CH_2)_3$ | *t*-BuMgCl | 89 |
| 8 | Bn | $Ph(CH_2)_3$ | *i*-PrMgCl | 62 |
| 9 | Bn | $CH_2=CHCH_2$ | MeMgBr | 43 |
| 10 | Bn | $CH_2=CHCH_2$ | EtMgBr | 43 |
| 11 | Bn | $CH_2=CHCH_2$ | *t*-BuMgCl | 69 |
| 12 | Bn | $CH_2=CHCH_2$ | *i*-PrMgCl | 99 |
| 13 | Me | $Ph(CH_2)_3$ | MeMgBr | 95 |
| 14 | Me | $Ph(CH_2)_3$ | EtMgBr | 71 |
| 15 | Me | $Ph(CH_2)_3$ | *t*-BuMgCl | 79 |
| 16 | Me | $Ph(CH_2)_3$ | *i*-PrMgCl | 83 |
| 17 | *t*-Bu | $Ph(CH_2)_3$ | EtMgBr | 85 |

As shown in the table the methodology resulted to be versatile by combining three amines and five Grignard reagents.

A one-pot procedure starting from amines was also optimized (**scheme 18**). The desired amine was treated with an alkoxy carbonyl chloride in the presence of triethylamine, after that the corresponding Grignard reagent was added and the amide was obtained in high chemical yield illustrated in **table 10**.

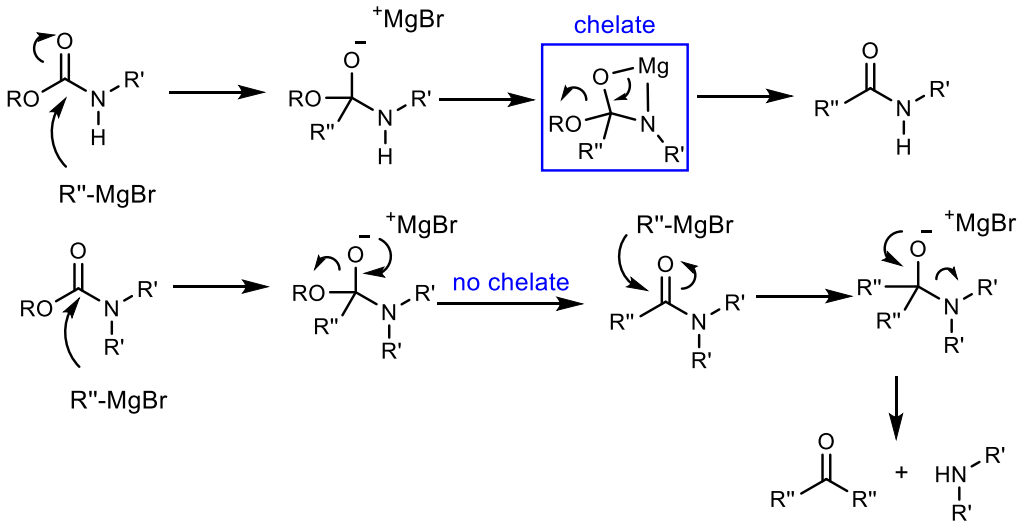



**Scheme 18**: One-pot conversion

**Table 10: One-pot procedure**

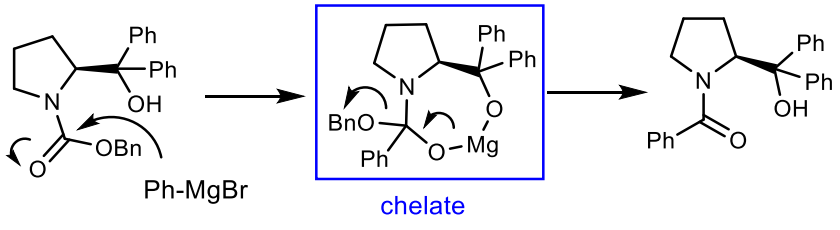
| Entry | $R_1$ | $R_2$ | $R_3$ | Yield (%) |
|---|---|---|---|---|
| 1 | $Ph(CH_2)_3$ | Me | Et | 87 |
| 2 | $Ph(CH_2)_3$ | Bn | Et | 92 |
| 3 | $Ph(CH_2)_3$ | Bn | Me | 66 |
| 4 | $CH_2{=}CHCH_2$ | Bn | Me | 70 |
| 5 | $CH_2{=}CHCH_2$ | Bn | *t*-Bu | 47 |
| 6 | $CH_2{=}CHCH_2$ | Bn | *i*-Pr | 58 |
| 7 | $CH_2{=}CHCH_2$ | Bn | Et | 36 |
| 8 | $CH_2{=}CHCH_2$ | Me | Et | 41 |
| 9 | $CH_2{=}CHCH_2$ | Me | Me | 83 |
| 10 | $Ph(CH_2)_3$ | *t*-Bu | Et | 90 |

When carbamates of secondary amines are used as a starting material instead of primary ones then corresponding ketone and secondary amine are obtained. It was conveniently reasoned an *N,O*-magnesium chelate intermediate is formed when primary amines are used (**scheme 19**).




**Scheme 19**: Mechanisms of the reactions

In the case of N-protected-diphenyl-L-prolinol a chelate can also be formed due to the presence of tertiary alcohol (**scheme 20**), following the primary amines behavior across a chelate intermediate.



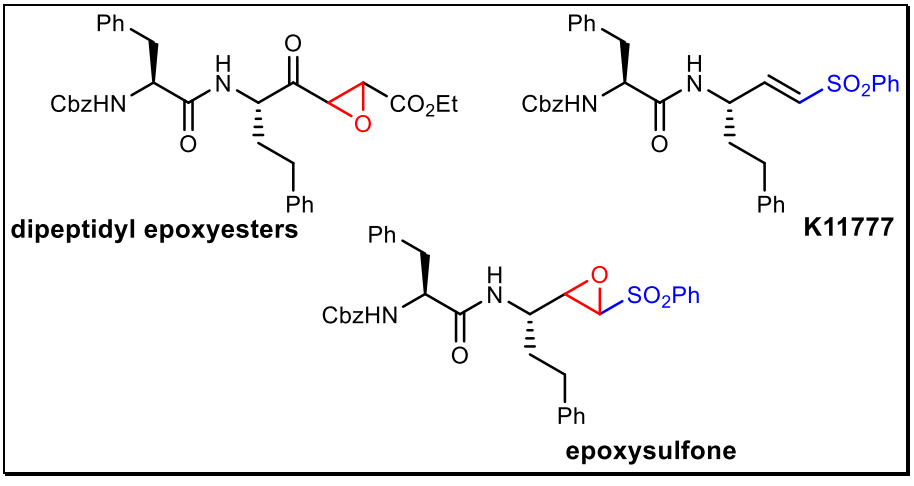

**Scheme 20**: Chelation intermediate for N-protected-diphenyl-L-prolinol

### 2.2.2. Conclusion

A new robust, versatile and cheap procedure was developed to transform carbamates into amides. Furthermore a one-pot procedure was optimized for the transformation of amines into amides using carbamates as intermediates. The wide range of amines and Grignard reagent showed the large number of products that could be obtained.

## 2.3. Epoxysulfones derived from amino acids as eletrophilic traps

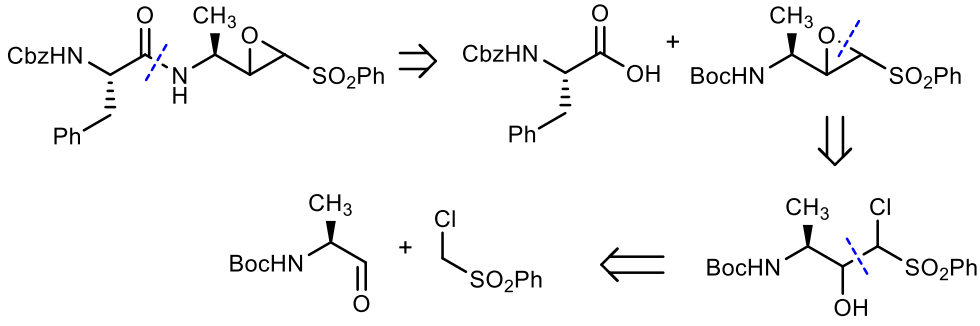
As mentioned before, epoxides are present in the structure of potent inhibitors of cysteine proteases such as E-64 and dipeptidyl epoxyesters. In the other hand, vinyl sulfones such as K11777 are also potent cysteine protease inhibitors. By combining both moieties, an epoxysulfone results as shown in **scheme 21**.




**Scheme 21**. Design of new inhibitors

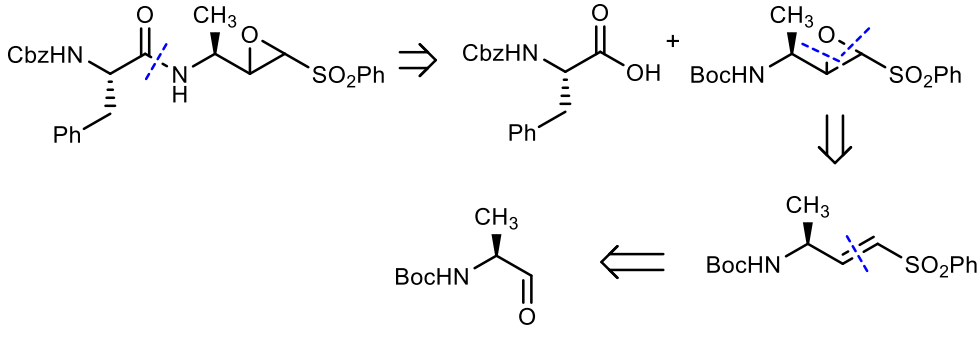
We planned to synthesize epoxysulfones as potential cysteine proteases inhibitors. The retrosynthetic route was designed considering two possible pathways; the Darzens route in **scheme 22** or the vinyl sulfone epoxidation in **scheme 23**.

Darzens route:




**Scheme 22**. Darzens route

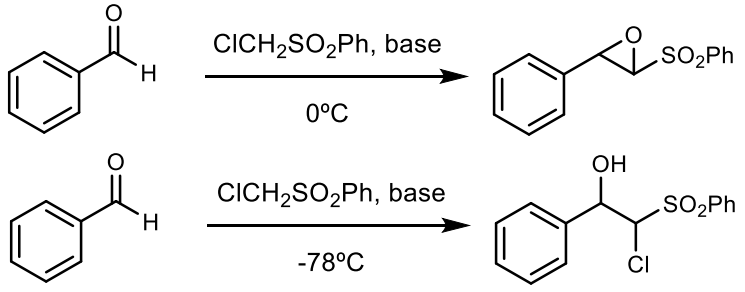
Vinyl sulfone epoxidation:




**Scheme 23**. Vinyl sulfone epoxidation route

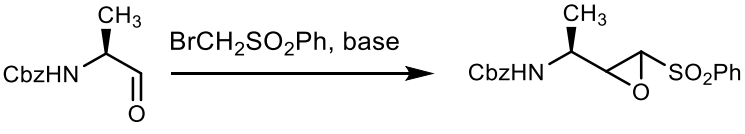
### 2.3.1. Darzens route

After a literature search, it was found that Darzens reactions to prepare epoxysulfones had been reported using aromatic aldehydes only.[119] The reactions were performed using aromatic aldehydes, chloromethyl phenyl sulfone and a base (KOH, *t*-BuONa…). Usually the reaction product was the epoxysulfone, only if the reaction is performed at -78 ºC halohydrins can be isolated.




**Scheme 24**. Reported Darzens conditions

The work was started by applying these previous reported conditions using carbobenzyloxy alaninal (1 equivalent) and commercially available bromomethylphenylsulfone (1 equivalent) as starting materials in the presence of a base (1 equivalent), shown in **scheme 25**. A range of bases and conditions were assayed in order to find optimal conditions are summarized in **table 11**.




**Scheme 25**. Reported Darzens conditions

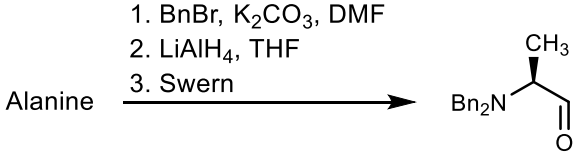
[119] Voght, P.; Tavares, D. *Can. J. Chem.* **1969**, *47*, 2875-2881

**Table 11. First Darzens condition test**

| Entry | Base | Conditions | Result |
|---|---|---|---|
| 1 | NaOH / TBABr | DCM/r.t. | SM |
| 2 | *t*-BuONa | *t*-BuOH/$Et_2O$/r.t. | SM |
| 3 | LDA (0.7eq) | THF/-78ºC | SM |
| 4 | LDA (1eq) | THF/-78ºC | SM |

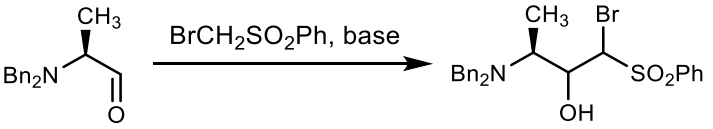
SM = starting material recovered

In all cases starting material was recovered as indicated in the **table 11**. Then it was reasoned that the NH group might be consuming base and the reaction did not work. For this reason it was decided to diprotect the amine group as a dibenzyl amine (**scheme 26**). Diprotection and esterification of alanine was carried out using an excess of benzyl bromide and potassium carbonate in dimethylformamide. A reduction of ester to a primary alcohol was carried out using lithiumaluminum hydride in tetrahydrofuran and a final Swern oxidation was done to afford to the desired diprotected aminoaldehyde.




**Scheme 26**. Preparation of N,N-protected aldehyde

Over this diprotected aldehyde several Darzens conditions were tested (**scheme 27**). Using kinetic bases (LDA and *n*-BuLi), four bromohydrins were detected in low yields (<20%) in about 25% for each diastereoisomer. Other bases as potassium hydroxide and sodium *terc*-butoxide did not afford to the desired epoxide, as it is shown in **table 12**.




**Scheme 27**. Darzens of diprotected aldehyde

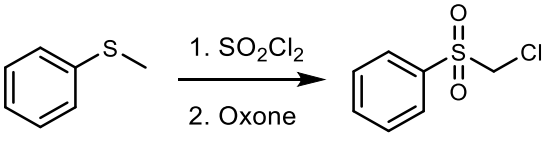
**Table 12. Darzens condition using diprotected alaninal**

| Entry | Base (1eq) | Conditions | Result |
|---|---|---|---|
| 1 | LDA | THF / -78ºC to r.t. | bromohydrins |
| 2 | KOH / TBABr | Tol / r.t. | SM |
| 3 | *t*-BuONa | *t*-BuOH / $Et_2O$ / r.t. | SM |
| 4 | *n*-BuLi | THF / -78ºC to r.t. | bromohydrins |

SM = starting material recovered

Although epoxides were not isolated, they were detected by NMR and then it was decided to isolate the halohydrins which could be used as intermediates to be further converted into desired epoxides.

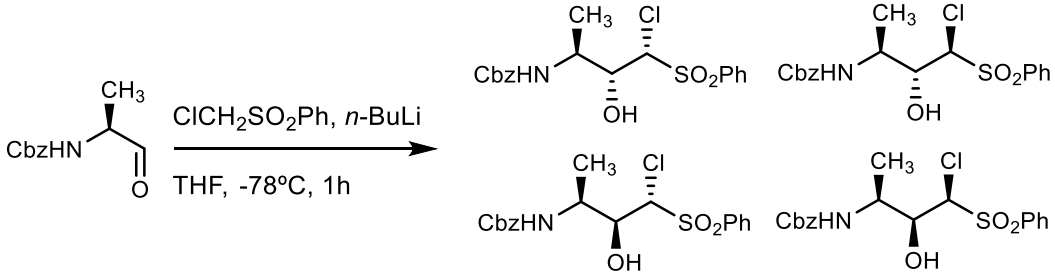
It was decided to assay the reaction using chloromethyl phenyl sulfone instead of bromomethyl phenyl sulfone. Chloromethyl phenyl sulfone was prepared in 92% yield from commercially available thioanisol with sulfuryl chloride in a first step and subsequent oxidation to sulfone with Oxone represented in **scheme 28**.[120]




**Scheme 28**. Preparation of chloromethyl phenyl sulfone

[120] Trost, B. M.; Grese, T. A. *J. Org. Chem.* **1991**, 56, 3189-3192

Darzens reactions were performed using carbobenzyloxy alaninal, chloromethyl phenyl sulfone and *n*-butyl lithium as a base, as shown in **scheme 29**. When one equivalent of base and chloromethyl phenyl sulfone were used starting material was recovered, then some reactions were performed with increasing number of equivalents of these two reagents. A satisfactory result was observed (**entry 4**) when 2.5 equivalents of chloromethyl phenyl sulfone and 2.5 equivalents of *n*-butyl lithium were added affording chlorohydrins in a 75 % chemical yield as a mixture of 4 diastereoisomers (about 30/26/23/21).

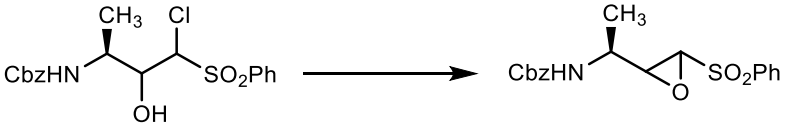



**Scheme 29**. Preparation of chloromethyl phenyl sulfone

**Table 13. Darzens condition using Cbz- alaninal**

| Entry | Base eq | Sulfone eq | Yield (%) |
|---|---|---|---|
| 1 | 1 | 1 | 0 |
| 2 | 1.5 | 1.5 | <15 |
| 3 | 2 | 2 | 65 |
| 4 | 2.5 | 2.5 | 75 |

The reaction crude was purified by chromatography, affording chlorohydrins as inseparable mixtures. The mixture of chlorohydrins was submitted to epoxidation conditions with different bases and solvents to obtain the epoxides, represented in **scheme 30**.



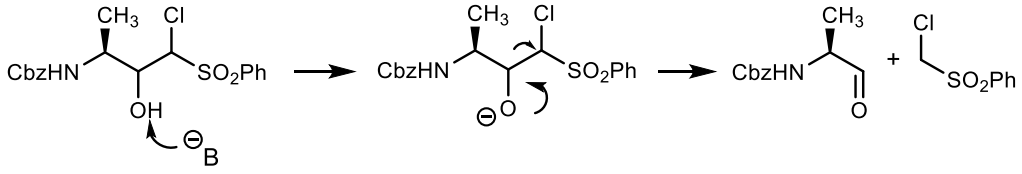

**Scheme 30**. Preparation of epoxides

**Table 14. Epoxidation of chlorohydrins**

| Entry | Base[a] | Solvent | Conditions | Result |
|---|---|---|---|---|
| 1 | $K_2CO_3$ | MeOH | 30 min / 0ºC | epoxides |
| 2 | NaH | THF | 30 min / 0ºC | retroDarzens |
| 3 | *t*-BuONa | THF | 30 min / 0ºC | retroDarzens |

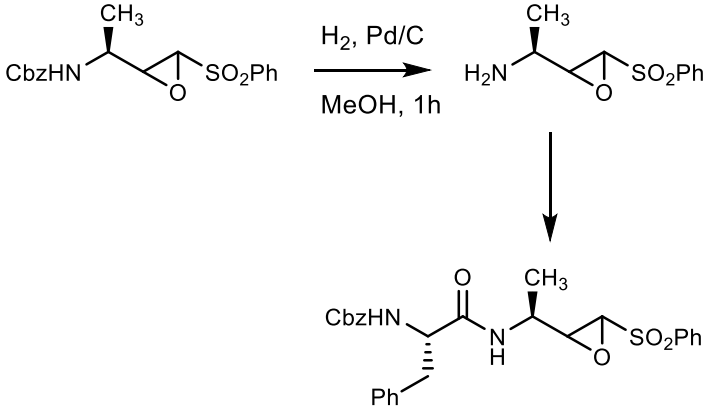
[a] 1 equivalent

As can be observed in the **table 14**, potassium carbonate in methanol (**entry 1**) afforded the epoxides as $^1$H-NMR and MS denoted but they could not be isolated by chromatography. When other bases were used (**entries 2-3**), retroDarzens mechanism took place, explained in **scheme 31**.




**Scheme 31**. RetroDarzens mechanism

The obtained epoxysulfones were used in the next step as a crude mixture to prepare desired dipeptidyl epoxysulfones. The deprotection of the amine and peptide coupling is shown in **scheme 32**. Deprotection of carbamate was assayed by hydrogenation reaction using palladium over charcoal as a catalyst under hydrogen atmosphere. After an extensive experimental work, no hydrogenation reaction assay afforded desired amine. Analysis of the reaction crude by NMR and MS denoted a complex reaction mixture of compounds. It was then decided to change the amine protecting group.

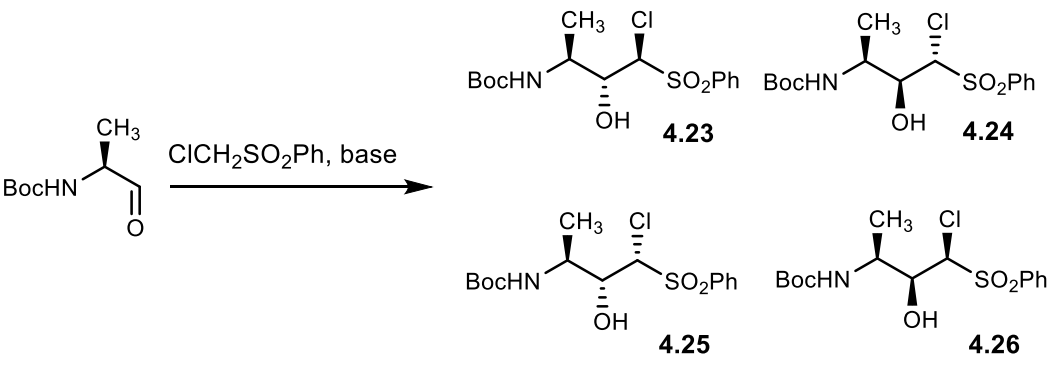



**Scheme 32**. Deprotection and coupling steps.

Due to the difficult to work with complex mixtures of chlorohydrins, the reaction crude without purification and the difficult to remove de protecting group

with the presence of the epoxide, it was decided to change to Boc group and try to isolate the chlorohydrins of Darzens reaction and study their configuration.

*tert*-Butoxycarbonyl alaninal was prepared by reduction of the ester or the Weinreb amide. Aldehyde was purified by chromatography before submitting it to Darzens reaction. This resulted to be critical in order to get a clean reaction mixture from the Darzens reaction, **scheme 33**. An extensive experimental work was performed to optimize Darzens reaction. Several Darzens conditions were tested and different results were obtained. A mixture of four chlorohydrins was obtained in all cases. Chromatographic separation of four isomeric chlorohydrins resulted to be a difficult task in some cases.




**Scheme 33**. Darzens reaction using Boc-alaninal

Table 15. Darzens results

| Entry | Base[a] | Conditions | Proportion[c] | Yield (%) |
|---|---|---|---|---|
| 1 | *n*-BuLi | THF, -78°C, 30min | 33:27:24:16 | 75 |
| 2 | *n*-BuLi | THF, -78°C, 5h | 35:26:25:14 | 77 |
| 3 | *n*-BuLi | THF, -90°C, 2h | 33:24:24:19 | 71 |
| 4 | *n*-BuLi | THF/DMF, -78°C, 30min | 27:15:46:12 | 66 |
| 5 | LDA | THF, -78°C, 30min | 36:29:21:13 | 70 |
| 6 | *t*-BuOK | THF, 0°C, 30min | degradation | - |
| 7 | *t*-BuOK | THF, -78°C, 30min | 17:8:42:33 | 55 |
| 8 | KHMDS | THF, -78°C, 30min | 40:13:29:17 | 63 |
| 9 | LiHMDS | THF, -78°C, 30min | 35:25:25:15 | 67 |
| 10 | *t*-BuOK | DMF, -78°C, 30min | Epoxides[b] (1/1.7) | 45 |

[a] 2.5 eq and 2.5 eq of chloromethyl phenyl sulfone

[b] *cis* (**4.29** + **4.30**) /*trans* ( **4.27** + **4.28**)

[c] of chlorohydrins (**4.23** : **4.24** : **4.25** : **4.26**)

Previous assays demonstrated that the reaction does not work if using less than 2 equivalents of base and 2 equivalents of sulfone. A satisfactory chemical yield was obtained when 2.5 equivalents of *n*-butyl lithium and 2.5 equivalents of chloromethyl sulfone were used affording a 33:27:24:16 mixture of chlorohydrins (**entry 1**). In this case the reaction was done in 30 minutes, tetrahydrofuran was used as a solvent and the temperature was -78 °C.

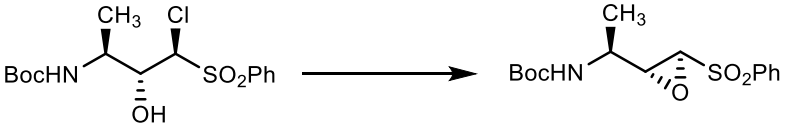
When reaction time and temperature were changed (**entries 2-3**) no improvement was observed. Other kinetic bases (LDA, KHMDS or LiHMDS) did not modify the standard result (**entries 5**, **8** and **9**). On the other hand when dimethylformamide was used as a co-solvent (**entry 4**) *syn* chlorohydrins were

isolated as major products reversely to the result obtained when using tetrahydrofuran as a single solvent of the reaction.

When sodium *tert*-butoxide was used as a base (**entry 7**), the *syn* / *anti* ratio of chlorohydrins changed dramatically (17:8:42:33) compared to kinetic lithium bases. If the reaction is carried out using sodium *tert*-butoxide at room temperature (**entry 6**) a complex mixture of unidentifiable products was observed for degradation trough the epoxide. Finally when using sodium *tert*-butoxide in dimethylformamide at 0 ºC, a 1.7/1 mixture of *cis* (**4.29** + **4.30**)/*trans* ( **4.27** + **4.28**)was observed (**entry 10**).

### 2.3.1.1. Epoxidation

The epoxidation reaction was the next step, represented in **scheme 34**. The first assays for the conversion of chlorohydrins into epoxides were carried out using chlorohydrin **4.23**. These conditions are summarized in next **table 16**. In all cases the reaction was performed using one equivalent of the base since it was observed some degradation of the reaction mixture when using more than one equivalent.




**4.23** **4.27**

**Scheme 34**. Epoxidation of chlorohydrin **4.23**

Table 16. General epoxidation conditions

| Entry | Base | Conditions | Observations |
|---|---|---|---|
| 1 | NaH | THF, 0ºC, 30min | retro + **4.25** |
| 2 | NaOH | DCM, 0ºC, 30min | degradation |
| 3 | $Et_3N$ | DCM, 0ºC, 30min | **4.23** |
| 4 | $K_2CO_3$ | MeOH, 0ºC, 1h | **4.27** + **4.23** |
| 5 | $K_2CO_3$ | THF, 0ºC, 1h | **4.27** + **4.25** |
| 6 | $K_2CO_3$ | DMF, 0ºC, 1h | **4.27** + retro |
| 7 | *t*-BuOK | THF, 0ºC, 1h | **4.27** + retro |
| 8 | *t*-BuONa | DCM, 0ºC, 30h | **4.27** |

retro = retoDarzens identified by presence of aldehyde and chloromethyl sulfone

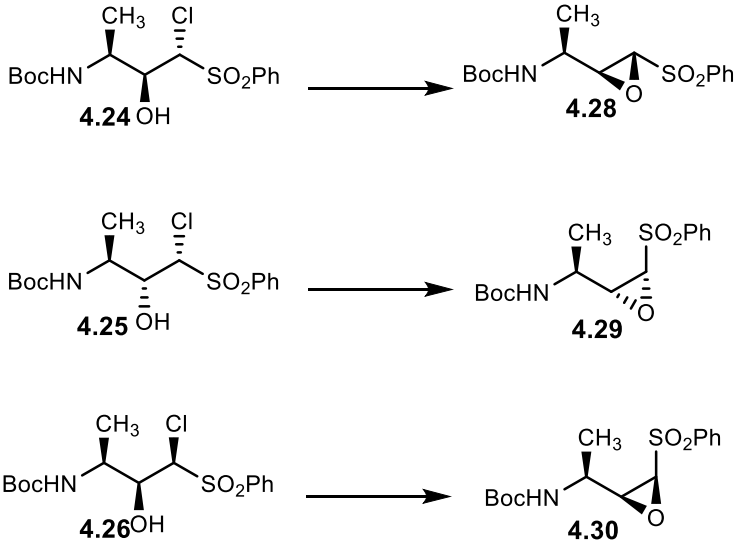
If triethylamine is used (**entry 3**) then starting material **4.23** was recovered. When sodium hydroxide (**entry 2**) was used then degradation was observed (aldehyde and and other byproducts), similar result was observed when potassium or lithium hydroxide were used.

If the reaction is carried out using sodium hydride then aldehyde, chloromethyl sulfone and chlorohydrin **4.25** were obtained, denoting retroDarzens and epimerization pathways. When potassium carbonate was used in methanol desired epoxide **4.27** was detected by NMR as a minor product although the main compound present in the reaction mixture was starting material **4.23**. Increasing reaction time and equivalents did not increase the conversion. Potassium carbonate in tetrahydrofuran gave rise to desired epoxide as a minor product and chlorohydrin **4.25** (**entry 5**). Dimethylformamide as a solvent gave rise to retroDarzens products and epoxide in a little amount (**entry 6**). Potassium *tert*-

butoxide in tetrahydrofuran afforded a significant amount of epoxide but the retroDarzens products were the main reaction products (**entry 7**).

Finally, sodium *tert*-butoxide in dichloromethane cold with an ice-bath afforded the desired epoxysulfone **4.27** as the single reaction product (**entry 8**). According to the coupling constants it was a *trans* epoxide (J = 1.4 Hz), confirming the *anti*-configuration of starting chlorohydrin **4.23**.

Same experimental conditions were applied to the other three chlorohydrins. Chlorohydrin **4.24** afforded *trans*-epoxide **4.28** as a single reaction product and chlorohydrins **4.25** and **4.26** afforded desired *cis*-epoxides **4.29** and **4.30** respectively, but also other byproducts were observed in the NMR of the crude mixture. Then for the preparation of *cis*-epoxides, initial reaction conditions were slightly changed by adding *tert*-butanol as a co-solvent. All transformations are summarized in **scheme 35**.

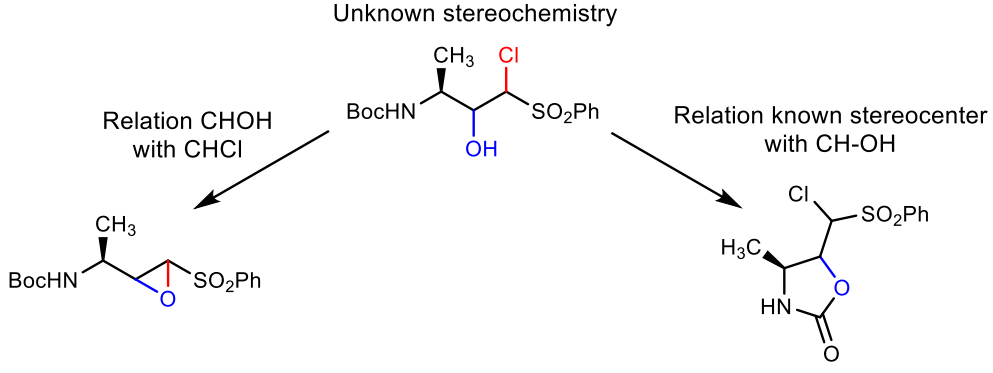



**Scheme 35**. Epoxides preparation

*cis*-Epoxides resulted to be quite unstable compounds (degradation was starting to be observed after a few hours standing on the bench). *trans*-Epoxides are less unstable than *cis* isomers (degradation is observed after a few days).

### 2.3.1.2. Stereochemistry confirmation

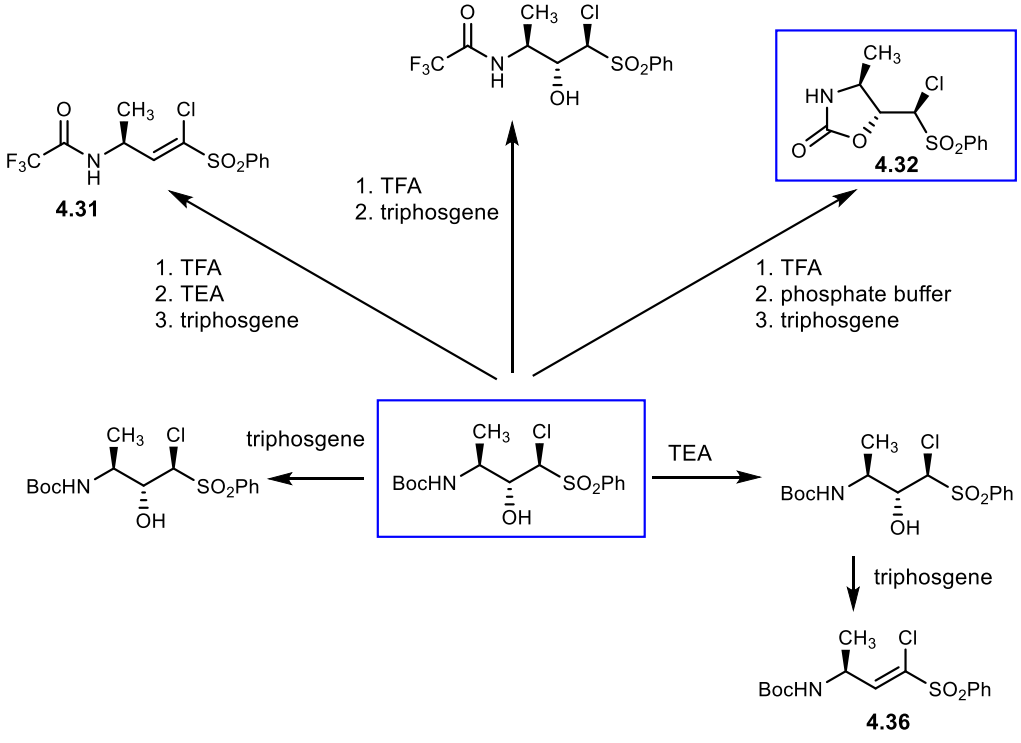
The stereochemistry of the chlorohydrins at the hydroxylated stereocenter was assigned by conversion into corresponding oxazolidinone after amine deprotection. In the other hand, configuration of the chlorinated stereocenter was assigned from the stereochemistry of the epoxide to be *cis* or *trans* (**scheme 36**).




**Scheme 36**. Assignment chlorohydrin **4.23**

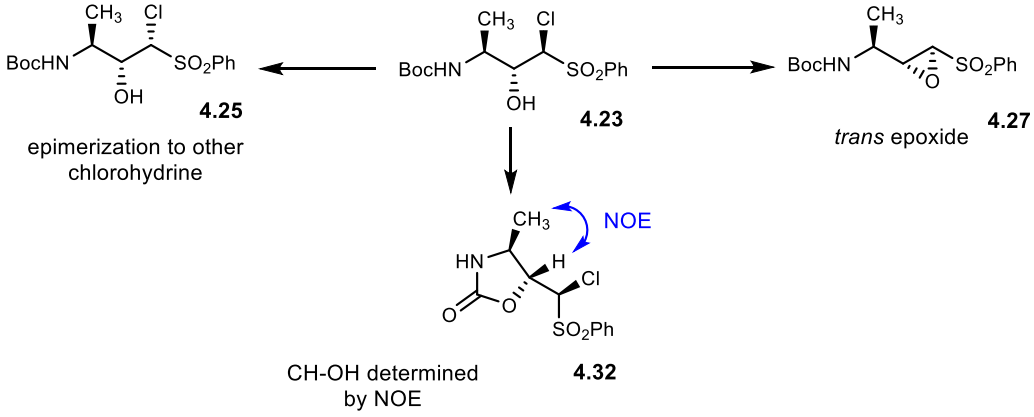
When chlorohydrin **4.23** was deprotected using trifluoroacetic acid and the resulting ammonium salt was directly submitted to reaction with triphosgene without any purification then undesired trifluoroacetamide was obtained. If ammonium salt resulting from deprotection was treated with triethylamine

previous to reaction with triphosgene, then a 1/1 mixture of E/Z chlorovinyl sulfones as trifluoroacetamides were obtained. Then it was decided to obtain the oxazolidinone without Boc deprotection. The chlorohydrin was directly treated with triphosgene, but starting material was recovered. On the other hand, when the N-protected chlorohydrin is treated with triethylamine and triphosgene then corresponding chlorovinyl sulfone **4.36** are formed.

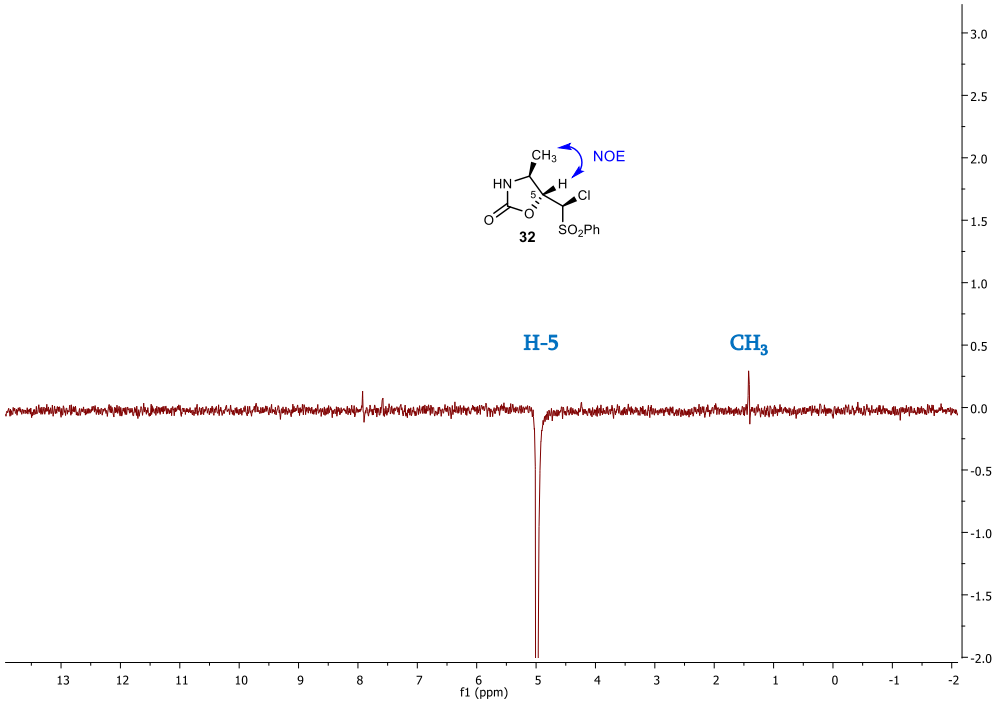



**Scheme 38**. Transformations during preparation of **4.32**

Finally it was decided to carry out an aqueous work up by extracting the amine resulting from Boc deprotection step with a phosphate buffer at pH 9. The resulting neutral amine was submitted to cyclization with triphosgene affording the desired oxazolidinone **4.32** and the structure can by assigned by NOE shown in **scheme 39** and **scheme 40**.

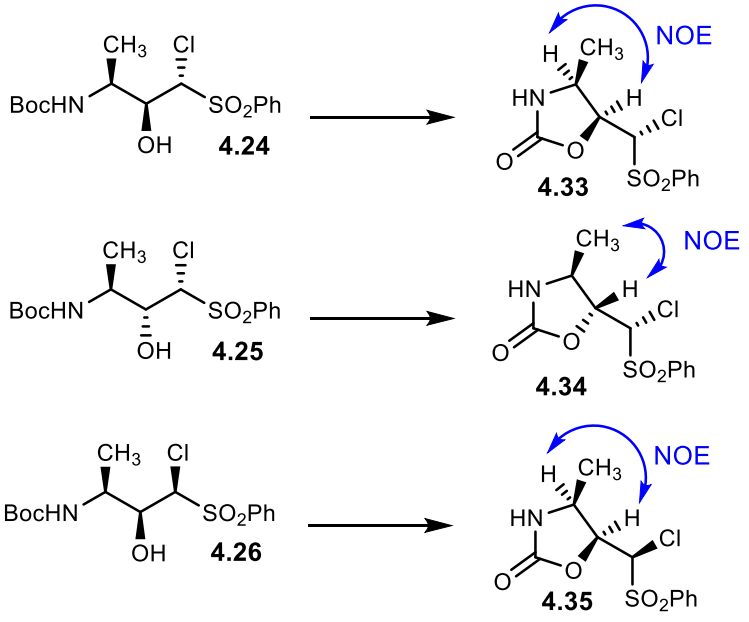



**Scheme 39**. Transformations for chlorohydrin **4.23**



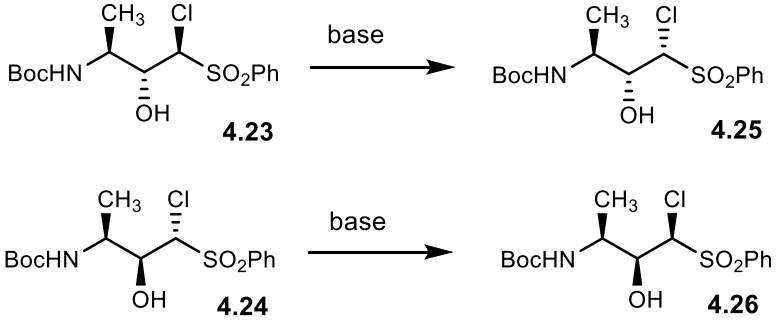

**Scheme 40**. NOE for oxazolidinone **4.32**

Stereochemistry of other three oxazolidinones from corresponding chlorohydrins was also assigned by NOE and summarized in **scheme 41**.



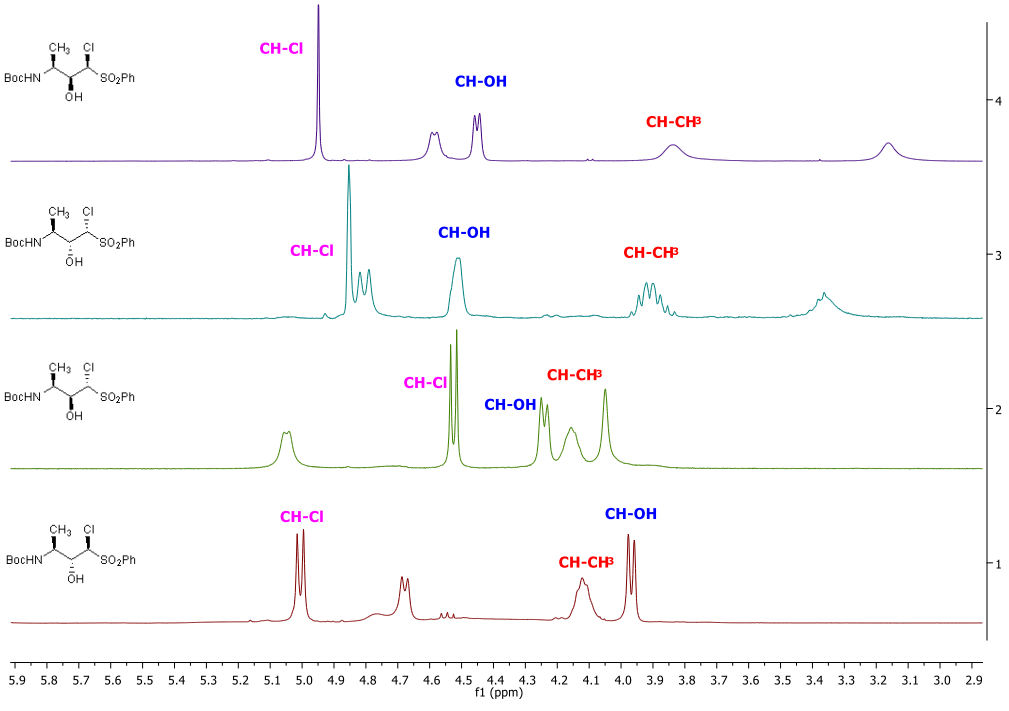

**Scheme 41**. NOE for oxazolidinones **4.33-4.35**

The conversion of chlorohydrin **4.23** into chlorohydrin **4.25** would result from epimerization of chlorinated stereocenter. In the same way chlorohydrin **4.24** is converted to chlorohydrin **4.26** through epimerization under basic conditions, shown in **scheme 42**, it afforded to a useful information about stereochemical relationship of chlorohydrins.




**Scheme 42**. Epimerization transformations for chlorohydrins **4.23-4.24**

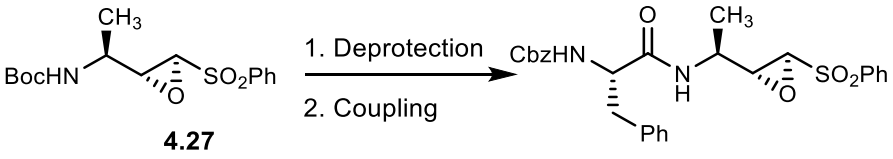
Some similarities are observed in the $^{1}$H-NMR spectra of the *anti*-chlorohydrins **4.23** and **4.24**, and it also happens between *syn*-compounds **4.25** and **4.26**. The CH-CH$_3$ signal in the *anti*-isomers is located around 4.15 ppm and in the case of *syn*-isomers appears at 3.85 ppm. The CH-OH signal for the *anti*-isomers is between 3.95 ppm - 4.25 ppm and for the *syn*-isomers is between 4.40 ppm - 4.55 ppm. Also for the coupling constants between CH-Cl and CH-OH some similarities are observed: for the *anti*-isomers **4.23** and **4.24** is 9.0 and 9.6 Hz, respectively; and for the *syn*-isomers **4.25** and **4.26** are 0 Hz for both cases.




**Scheme 43**. NMR similarities for chlorohydrins **4.23**-**4.26**

Once the preparation of the epoxysulfones derived from L-alanine was accomplished then their conversion into a modified dipeptide inhibitor was

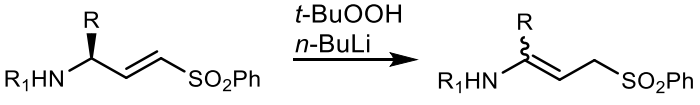
assayed. All the deprotection assays were not successful. This transformation are currently undergoing in our group.




**Scheme 44**. Transformation to dipeptide structure

### 2.3.2. Vinyl sulfone epoxidation route

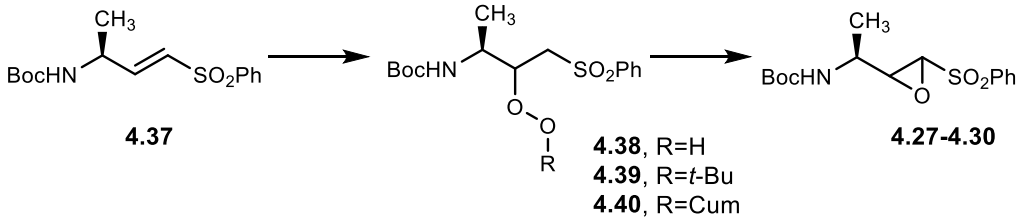
On the other hand, the preparation of epoxysulfones through epoxidation of vinylsulfones was also studied. It had been reported[121] that when carrying out a nucleophilic epoxidation over a vinyl sulfone then allyl sulfones are formed (*t*-BuOOH/*n*-BuLi). Some new reaction conditions were studied to carry out the desired epoxidation without the isomerization into allyl sulfones.




**Scheme 45**. Isomerization of vinyl to allyl sulfones

The reaction, **scheme 46**, was carried out with different bases and using different oxidants as summarized in **table 36**.

[121] Götz, M.G.; Caffrey, C.R.; Hansell E.; McKerrow J.H.; Powers, J.C. *Bioorg. Med .Chem.* **2004**, *12*, 5203–5211




**Scheme 46**. Epoxidation of vinyl sulfone

**Table 17. Epoxidation reaction**

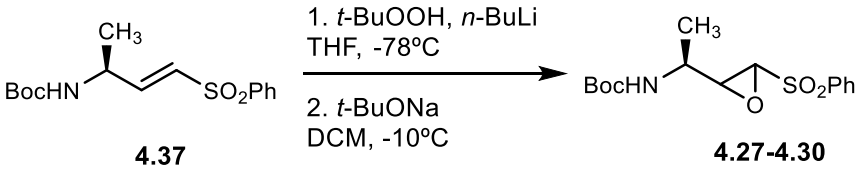
| Entry | Oxidant | Base | Conditions | Results |
|---|---|---|---|---|
| 1 | *m*-CPBA | - | DCM, r.t., 2days | **4.37** |
| 2 | *t*-BuOOH | Triton B | MeOH, 0ºC, 2h | **4.37** |
| 3 | *t*-BuOOH | NaOH | MeOH, 0ºC, 1h | **4.39**[a] |
| 4 | $H_2O_2$ | NaOH | MeOH, 0ºC, 2h | **4.38**[a] |
| 5 | Cum-OOH | NaOH | MeOH, 0ºC, 1h | **4.40**[a] |

[a] about 1:1 mixture of isomers

Peracid epoxidation did not work (**entry 1**). Same result was obtained when a combination of *tert*-butyl hydroperoxide and Triton B was used. But when a hydroperoxide (*t*-BuOOH, $H_2O_2$, Cum-OOH) and sodium hydroxide in methanol was used, a complete consumption of alkene was detected although the reaction product was the corresponding peroxyether **4.38**-**4.40** as a 1/1 mixture of non-isolable isomers.

Epoxysulfones can be obtained by treatment of the mixture of peroxides **4.38**-**4.40** with sodium *tert*-butoxide in dichloromethane at 0 ºC, affording the formation of the same four epoxides **4.27**-**4.30** prepared by the above explained Darzens reactions.

The transformation of vinylsulfones into epoxysulfones could be accomplished in one-pot as illustrated in **scheme 47**.

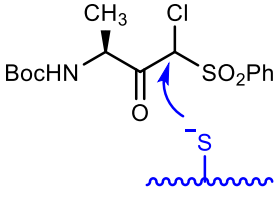



**4.37** **4.27-4.30**

**Scheme 47**. Epoxidation of vinyl sulfone in one-pot

### 2.3.3. Chloroketone sulfones as inhibitors

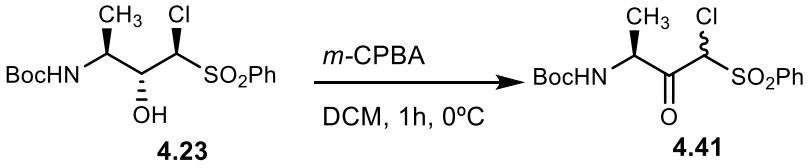
In order to transform chlorohydrins into other inhibitors besides epoxysulfones, oxidation of the hydroxyl group into the corresponding ketone was considered. It is known halomethylketones to be good inhibitors of cysteine protease[122] so the presence of a sulfone could increase de activity (**scheme 48**).




**Scheme 48**. Cysteine thiol attacks to chloroketones

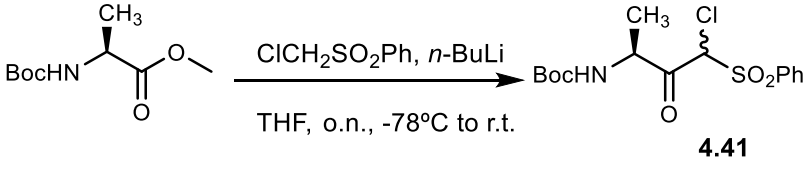
Chlorohydrin **4.23** was treated with *meta*-chloroperbenzoic acid in dichloromethane for 1h cold with an ice-bath. The reaction afforded a 1/1 mixture of isomeric sulfonyl chloroketones in high chemical yield (89%), as indicated in **scheme 49**.

[122] Rasnick, D. *Anal. Biochem.* **1985**, *149*, 461-465




**Scheme 49**. Preparation of chloroketones **4.41** from chlorohydrin **4.23**

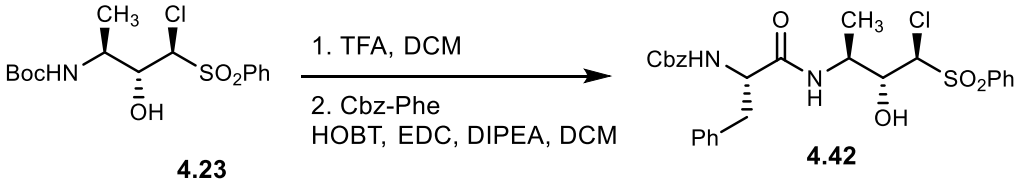
Another synthetic pathway to the ketone **4.41** was explored through addition of chloromethylsulfone anion to methyl ester. The chlorosulfone was ionized using *n*-buthyl lithium at -78ºC in tetrahydrofuran and after a few minutes ester was added in tetrahydrofuran. After stirring overnight at room temperature, chloroketones were obtained as 1/1 mixture of isomeric (chemical yield = 68%), illustrated in **scheme 50**.




**Scheme 50**. Preparation of chloroketones **4.41** from ester

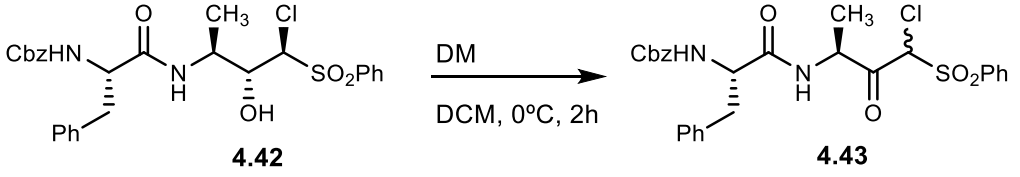
### 2.3.4. Related compounds

Chlorohydrin **4.23** derived from alanine has been also transformed into dipeptidyl chlorohydrin **4.42,** in **scheme 51**. Chlorohydrin was deprotected with trifluoroacetic acid and coupled with carbobenzyloxy-L-fenilalanine to afford the desired product in good chemical yield (85%).




**Scheme 51**. Preparation of chloroketones **4.42**

Dipeptidyl compound **4.42** was oxidized with Dess-Martin periodinane to α-chloro β-keto sulfone **4.43** (chemical yield = 75%) as a 1/1 diastereoisomeric mixture represented in **scheme 52**.



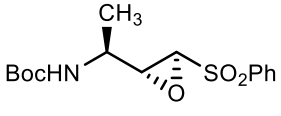

**Scheme 52**. Preparation of chloroketones **4.43** from ester

Dipeptidyl chlorohydrin **4.42** it was submitted as last chance to obtain dipeptidyl epoxysulfones, applying same conditions used before, but only epimerization and retroDarzens compounds could be detected by NMR and MS spectra.

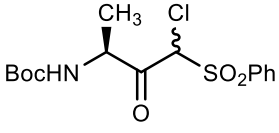
### 2.3.5. Inhibition testing

These assays were carried out during my research stay at Leiden University under supervision of Prof. Dr. Herman S. Overkleeft and Dr. Bogdan I. Florea.

Compounds **4.27** and **4.41** were investigated for their ability to inhibit cathepsins by using a competitive activity-based protein profiling (ABPP) assay against the known cysteine protease cathepsin activity-based probe DCG04-BodipyFL. Cathepsins from mouse liver lysates were treated with compounds **4.27** and **4.41**, and the residual cathepsin activity was captured by the DCG04-BodipyFL probe. After SDS-PAGE separation and in-gel fluorescence imaging, a typical band pattern was seen, which was compared with reported activity-based cathepsin profiling gels. By comparison of the molecular weights of the individual bands seen, we have assigned the nature of the individual cathepsins labeled by DCG04-BodipyFL. Competition for most of the bands was observed upon cotreatment with DCG04-BodipyFL, a result that underscores the annotation of the bands as being DCG04-BodipyFL sensitive cathepsins. Compound **4.27** is a more selective inhibitor than DCG04-BodipyFL, as it inhibits one to three cathepsins, while compound **4.41** inhibits four cathepsins at the 1 μM concentration used in this assay. Compounds **4.27** and **4.41** are able to outcompete the DCG04-BodipyFL inhibitor, and we can conclude that they bind in a covalent and irreversible fashion to the cathepsins, inhibiting their activity.

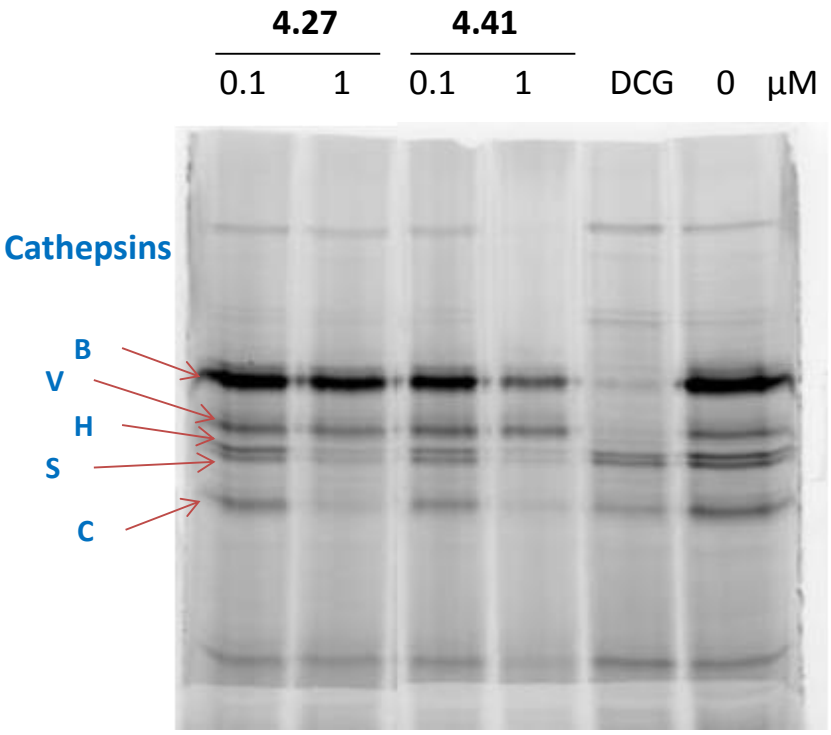



**4.27**




**4.41**

**Scheme 53**. Structure of tested inhibitors



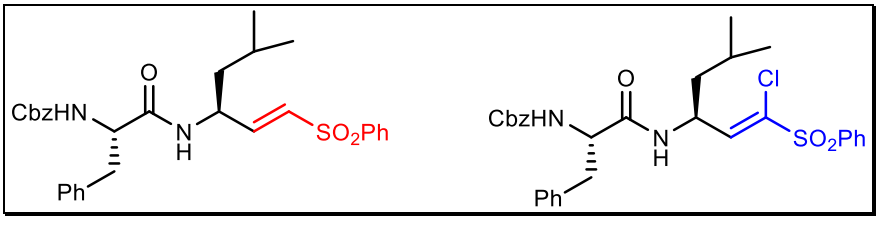

**Scheme 54**. ABPP for compounds **4.27** and **4.41**

These results demonstrate epoxysulfones and sulfonyl chloroketones to be promising electrophilic traps for cathepsins inhibitors. In both cases, they was good inhibitor a relative low concentrations. It could be increased modifying the amino acid structure to increase the affinity in the active site.

On the other hand, an interesting selectivity in both cases with the different cathepsins should be mentioned. Cathepsins H, S and C was selectively inhibited in presence of cathepsins B and V, for this reason the development of this new traps are interesting.

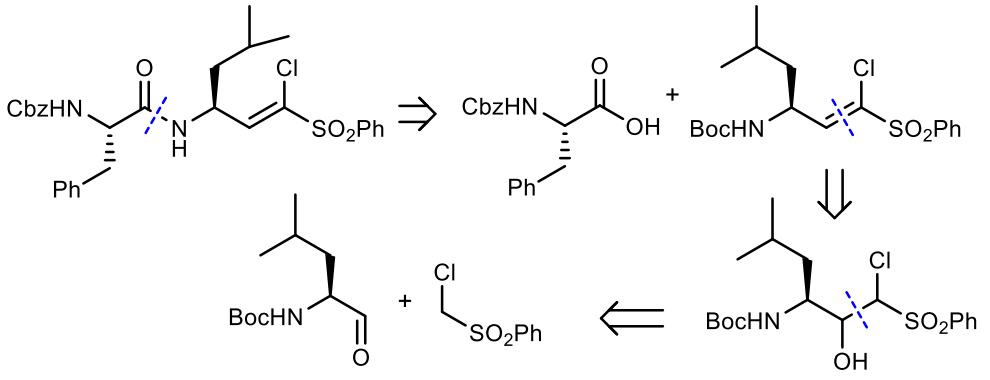
## 2.4. Chlorovinyl sulfones as new inhibitors

In the previously mentioned **scheme 38**, we isolate the chlorovinyl sulfone **4.36** during the attempt to prepare the oxazolidinones. We decided to take advantage of this result and prepare chlorovinyl sulfones as new inhibitors of cysteine proteases because they are very similar to the already known vinyl sulfones.



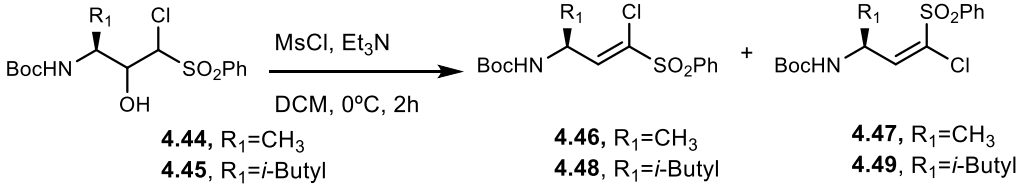

**Scheme 55**. Vinyl sulfone and chlorovinyl sulfone inhibitors

The retrosynthetic route was based on the chlorohydrins as previously explained for Darzens methodology**, scheme 56**.




**Scheme 56**. Retrosynthesis of chlorovinyl sulfones

The synthesis started with a mixture chlorohydrin derived from leucine and alanine. It were submitted to a elimination using mesyl chloride and a catalytic amount of triethylamine in dichloromethane at 0ºC for 2h (**scheme 54**), isolating by column as about 1/1 (E/Z) isomers in excellent yields **4.46** (45%), **4.47** (41%), **4.48** (37%) and **4.49** (32%).



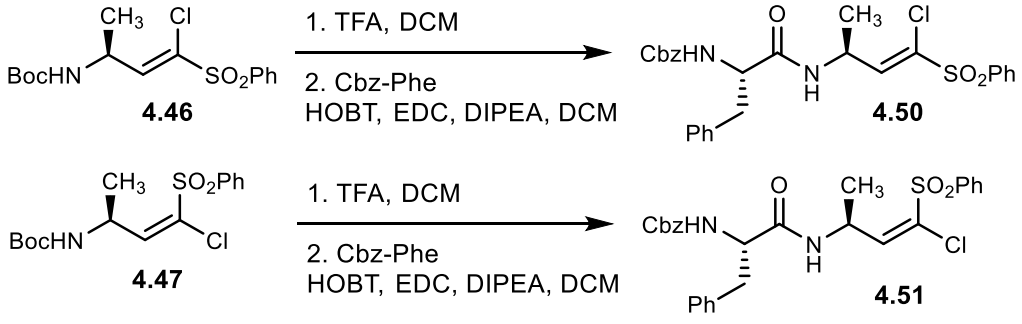

**4.44,** $R_1$=$CH_3$
**4.45**, $R_1$=*i*-Butyl

**4.46,** $R_1$=$CH_3$
**4.48**, $R_1$=*i*-Butyl

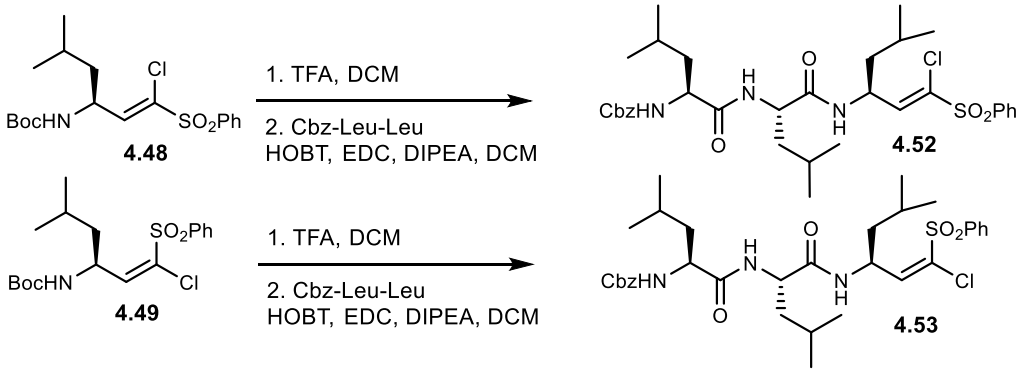
**4.47,** $R_1$=$CH_3$
**4.49**, $R_1$=*i*-Butyl

**Scheme 57**. Preparation of chlorovinyl sulfones

A deprotection of Boc group with trifluoroacetic acid in dichloromethane and a coupling with desired amino acid in dichloromethane for overnight afforded to the desired dipeptidyl compound **4.50** (81%) and **4.51** (85%) in **scheme 58** or tripeptidyl compound **4.52** (72%) and **4.53** (65%) in **scheme 59**.




**Scheme 58**. Preparation of dipeptidyl chlorovinyl sulfones



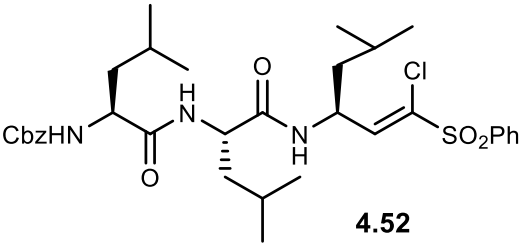

**Scheme 59**. Preparation of tripeptidyl chlorovinyl sulfones

### 2.4.1. Inhibition testing

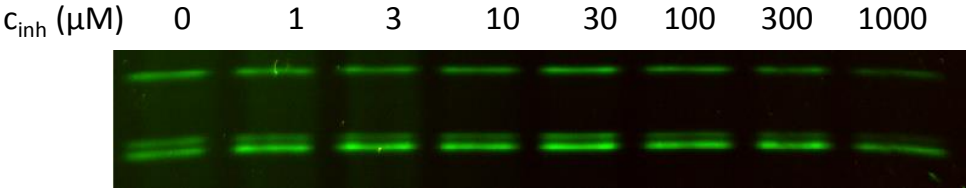
*In vitro* testing against cysteine proteases are currently carried out and the related publication is in progress.

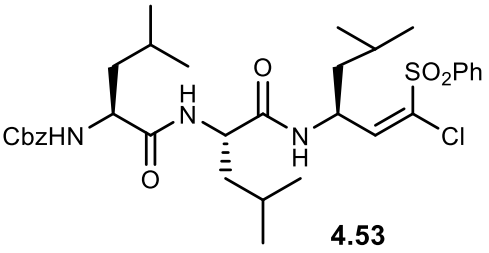
Biological test in Hela cell lysate

The standard protocol was carried out with 1h of incubation, 1h labelling of residual activity with LWA-300 (green bodipy-epoxomicin) and resolve on 12.5 SDS-PAGE.

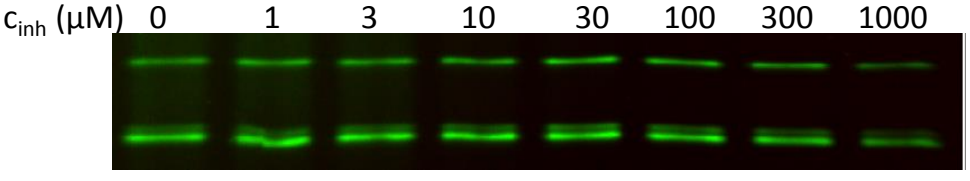



4.52




4.53

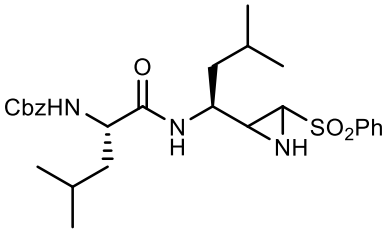



**Scheme 60**. Inhibition results for compounds **4.52**-**4.53**

The presence of a Cl in the α-position of the sulfone is decreasing the inhibition activity. This change is probably explained by the big steric hindrance caused by the chlorine atom close to the reactive carbon in β-position. This difficulty to attack is decreasing the inhibition up to 1000 μM of inhibitor concentration.

## 2.5. New sulfonyl-aziridines as inhibitors

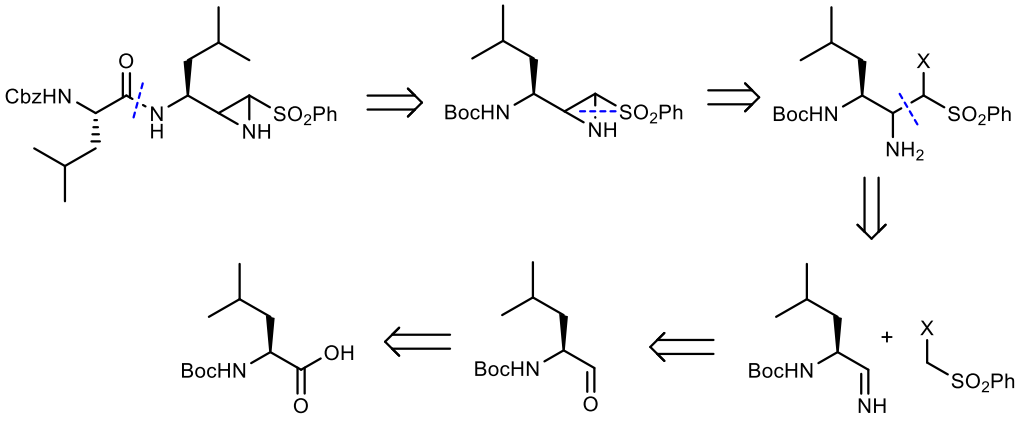
This work was done in my research stay at University of Leiden, in the Institute of Chemistry under Pr. Dr. Hermen Overkleeft supervision. The goal of this project was developed a methodology to afford to new sulfonylaziridines to check the activity against cathepsins based on the aziridines studies of Schirmeister et al.[123]




**Scheme 61**. Sulfonyl aziridine proposed

Retrosynthesis of this inhibitor was based upon an aza-Darzens reaction as a key step:

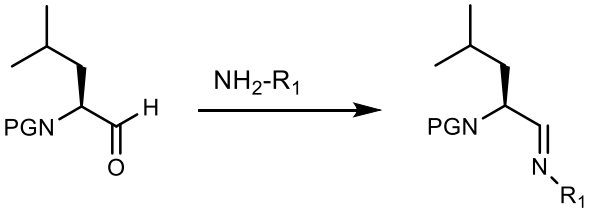
[123] Schirmeister, T. *J. Med. Chem.* **1999**, *42*, 560




**Scheme 62**. Retrosynthetic route to aziridines

The first step of the synthesis was obtaining the imine derivative from the desired amino acid.

The limiting factor was the nature of the substituent due to the imine formed should be reactive for the nucleophilic attack of the chloromethylphenyl sulfone. In this case it was tried with phenylsulfonamide in different experimental conditions.




**Scheme 63**. Preparation of imine intermediate

**Table 37. Imine preparation**

| Entry | PG | Amine, $R_1$ | Reagent | Solvent | T/t | Result |
|---|---|---|---|---|---|---|
| 1 | Boc | $SO_2Ph$ | $MgSO_4$ | DCM | 0 / 4h | SM |
| 2 | Boc | $SO_2Ph$ | $MgSO_4$ | DCM | 0 / 2days | SM |
| 3 | Boc | $SO_2Ph$ | $Et_3N$ | DCM | 0 / 4h | byP+ SM |
| 4 | Boc | $SO_2Ph$ | $BF_3$ | DCM | 25 / o.n. | byP |
| 5 | Boc | $SO_2Ph$ | $MgSO_4$ | THF | 0 / 4h | SM |
| 6 | Boc | $SO_2Ph$ | $Ti(O\textit{i}\text{-}Pr)_4$ | DCM | 0 / 4h | byP + SM |
| 7 | Boc | $S(O)SO_2Ph$[a] | - | DCM | 0 / 2h | SM |
| 8 | Boc | $S(O)SO_2Ph$[a] | $MgSO_4$ | DCM | 0 / 24h | byP + SM |
| 9 | $Boc_2$ | $SO_2Ph$ | $MgSO_4$ | DCM | 0 / 4h | SM |
| 10 | $Boc_2$ | $SO_2Ph$ | $Et_3N$ | DCM | 0 / 4h | byP + SM |
| 11 | $Boc_2$ | $SO_2Ph$ | $BF_3$ | DCM | 25 / o.n. | byP |
| 12 | $Boc_2$ | $SO_2Ph$ | $Ti(OiPr)_4$ | DCM | rt / o.n. | byP |

SM = starting material were recovered

byP = unidentified by products were detected

a = prepared refluxing phenhylsulfonamide with thionyl chloride

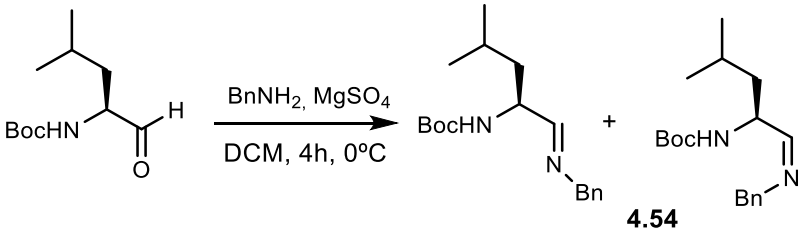
Phenyl sulfonamide was not enough nucleophile to attack the aldehyde generated, and then activation was required. In basic conditions (**entry 3**) starting material were recovered and the presence of unknown minor byproducts were detected. Activation with magnesium sulfate in dichloromethane or tetrahydrofuran (**entries 1-2**) only starting materials were isolated in perfect conditions. When boron trifluoride was used, dirtier crude spectra was detected and the presence of starting materials amounts was observed. Finally activation

using titanium tetra *iso*-propoxide did not afford to the desired product. Only starting material and unknown products were detected in the crude reaction.

Same results were obtained when phenylsulfonamide derivative (readily obtained by treatment of phenylsulfonamide with thionylchloride) was used (**entries 7-8**), only starting materials and byproducts were detected.

Finally the results when the aldehyde was diprotected with two Boc groups, the results are the same as for the analogous one having one Boc group (**entries 9-12**). For this reason it was decided to change to a more nucleophilic amine such as benzylamine.

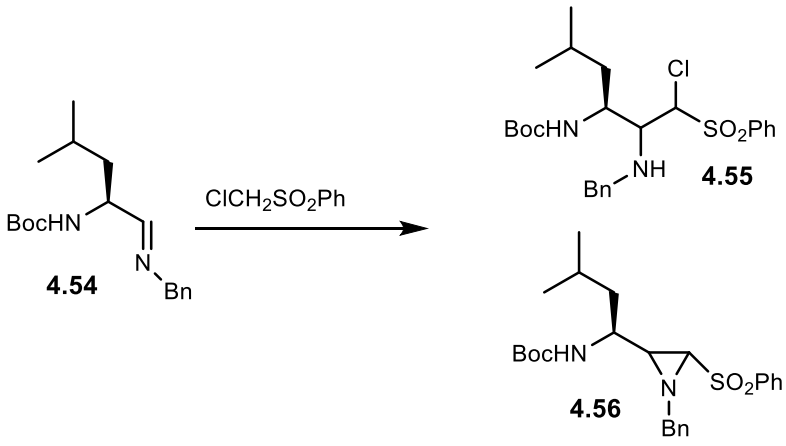
The imine was formed in good chemical yield (80% as calculated by NMR) using magnesium sulfate as a catalyst in dichloromethane cold with an ice bath for 4h, based on Palomo's work.[124] According to the NMR, imine **5.54** is an isomeric mixture in 92:8 ratio (**scheme 64**). The imine reaction crude was very pure and used in the next step without any further purification.




**Scheme 64**. Preparation imines **4.54**

[124] Palomo C., Cossio F.P., Cuevas C., Lecea B., Mielgo A., Roman P., Luque A., Martinez-Ripoli M. *J. Am. Chem. Soc.*, **1992**, *114* , 9360–9369

The aza-Darzens addition of chloromethylphenyl sulfone to this imine **4.54** was carried out represented in **scheme 62**.




**Scheme 62**. Preparation aziridines **4.56**

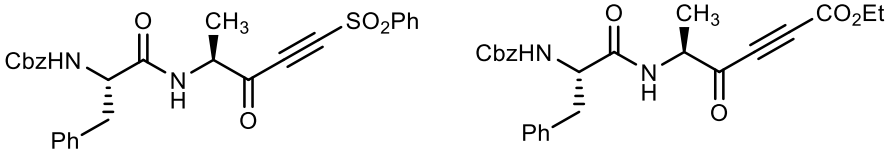
**Table 38. Preparation aziridine 4.56**

| Entry | Base | Solvent | T/t | Result |
|---|---|---|---|---|
| 1 | *n*-BuLi | THF | -78ºC / 4h | **4.55** + **4.56** |
| 2 | *n*-BuLi | THF | -78ºC to 0 ºC / 6h | **4.55** + **4.56** |
| 3 | *n*-BuLi | THF | -78ºC to r.t. / o.n. | **4.55** + **4.56** |
| 4 | LHMDS | THF | -78ºC / 4h | **4.55** + **4.56** |
| 5 | *t*-BuOK | DCM | 0 ºC / 4h | **4.55** + **4.56** |

The experimental procedure started with deprotonation of chloromethyl phenyl sulfone using a base and then the imine was added in the corresponding solvent. In all cases a mixture of aziridine and chloro-amine was detected by HPLC-MS of the crude mixture. NMR experiments of the crude mixture did not allow characterization of aziridine nor chloro. No isolated product was obtained after chromatography. More experiments to optimize this reaction need to be done. It will be tested and publish in an organic or medicinal journal.

## 2.6. Dipeptidyl carbonyl acetylenic inhibitors

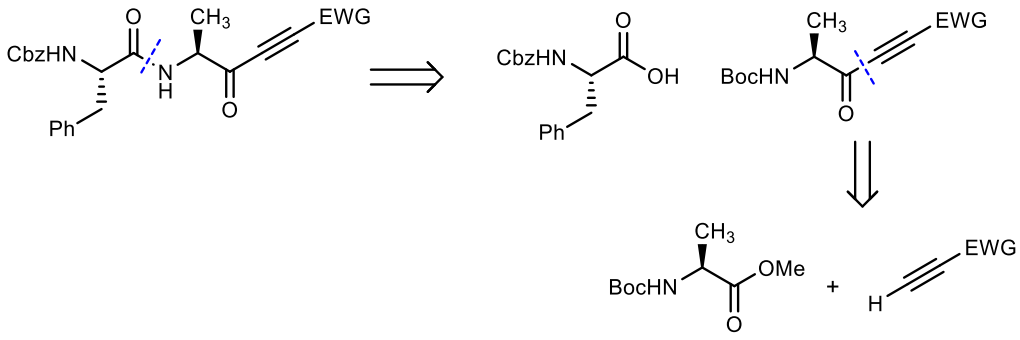
In order to explore new warheads for cysteine proteases inhibitors and encouraged by the high inhibition results of dipeptidyl enones reported recently by our group,[125] new acetylenic inhibitors were designed:




**Scheme 63**. Designed acetylenes

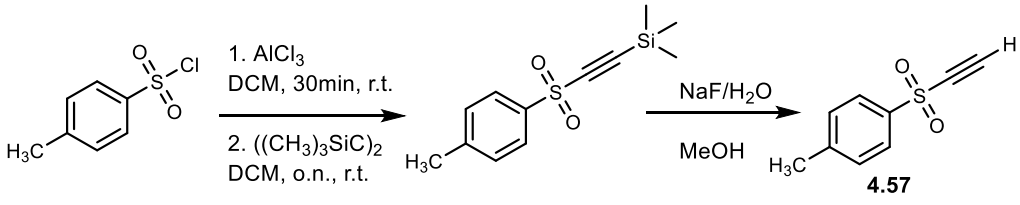
[125] Royo, S.; Rodriguez, S.; Schirmeister, T.; Kesselring, J.; Kaiser, M.; Gonzalez, F. *Chem. Med. Chem* **2015**, 10, 1484 –1487

The retrosynthetic analysis for these inhibitors is described below



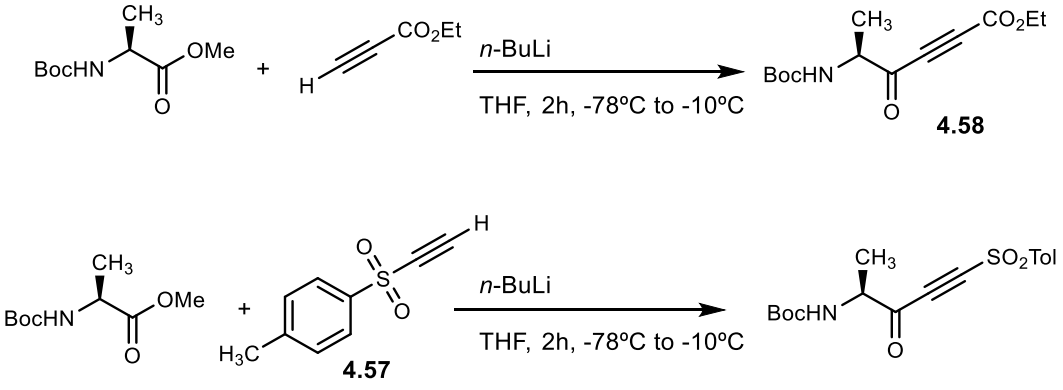

**Scheme 64**. Retrosynthesis of acetylenic inhibitors

The ethinyl *p*-tolyl sulfone was prepared from *p*-toluensulfonyl chloride in two step in good yield.



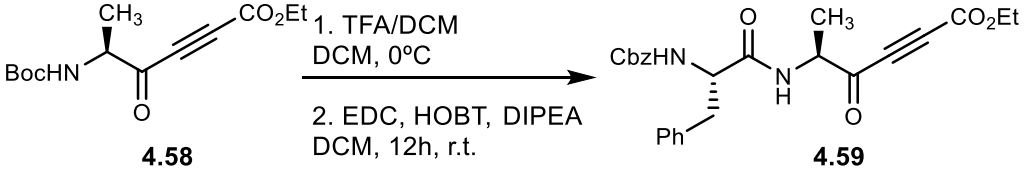

**Scheme 65**. Preparation ethinyl *p*-tolyl sulfone

Commercial ethylpropiolate and ethinyl *p*-tolyl sulfone were treated with *n*-butyl lithium in tetrahydrofuran at -78 ºC for 20 minutes and then ester was added in tetrahydrofuran and then 2h at -10 ºC. It was isolated acetylene **4.58** after column in moderated yield (26%) but no evidence sulfone derivate was detected. Several reaction conditions was tested but no product was formed.




**Scheme 66**. Preparation acetylenic inhibitors

Then deprotection of the carbamate group of **4.59** was carried out using trifluoroacetic acid in dichloromethane. Subsequent coupling with Cbz-Phe in dichloromethane afforded the desired dipeptidyl compound in excellent chemical yield (94%).




**Scheme 67**. Preparation acetylenic inhibitor **4.59**

*In vitro* testing for this compound is currently being carried out and it will be publish in a medicinal journal.

# 3. Experimental information

### Competitive Activity-Based Profiling of Cathepsins in Mouse Liver Lysates

Mouse liver was obtained from a Balb/c mouse (in conformity with the Leiden University animal experimentation protocol 13191u), cut into ca. 100 mg pieces, and frozen in liquid $N_2$. For lysate production, the liver segment was taken up in 3× volumes of ice-cold lysis buffer (50 mM MES, pH 5.0, 250 mM sucrose, 0.025% digitonin) for 30 min on ice and disrupted 10 times with 15 s sonication pulses interrupted by 5 s breaks on ice. The insoluble fraction was pelleted by centrifugation at 14 000 rpm in a cooled Eppendorf centrifuge, the supernatant was collected, and the protein concentration was determined with the Bradford (BioRad) colorimetric method using a BSA calibration curve. For competitive activity-based profiling, some 20 μg of total protein in 8 μL of lysis buffer at pH 5.0 was first incubated by addition of 1 μL of 10× solution of the indicated inhibitor concentration for 30 min at 37 °C, followed by addition of 1 μL of a 5 μM solution of the broad-spectrum cathepsin activity-based probe (ABP) DCG04-BodipyFL to label the residual cathepsin activity for 30 min at 37 °C. Nonfluorescent DCG04 cathepsin inhibitor at a final concentration of 1 μM was used as a positive control.

To assess the total labeling pattern of the DCG04-BodipyFL ABP, the lysate was first incubated with 1 μL of DMSO, and then samples were boiled with 6 μL of 4× Laemmli's sample buffer under reducing conditions and resolved for 2 h on a 12.5% SDS-PAGE gel. After rinsing with water, gel slabs were imaged with a Typhoon 2000 imager (GE Healthcare) using the fluorescein setting ($\lambda_{ex}$ = 490 nm, $\lambda_{em}$ = 510 nm). Gel images were acquired with ImageQuant and colored with ImageJ.

## 3.1. General Experimental Methods

All of the solvents used in reactions were freshly distilled from appropriate drying agents before use. $^{1}H$ and $^{13}C$ NMR spectra were measured in $CDCl_3$ ($^{1}H$, 7.24 ppm; $^{13}C$, 77.0 ppm) at 30 °C on a 300 or 500 MHz NMR spectrometer. IR spectra were recorded on oil films, KBr discs, or NaCl pellets on an FT-IR spectrometer. Mass spectra were measured in a QTOF I (quadrupole–hexapole TOF) mass spectrometer with an orthogonal *Z*-spray-electrospray interface. EM Science silica gel 60 was used for column chromatography, while TLC was performed with precoated plates (Kieselgel 60, $F_{254}$, 0.25 mm). Unless otherwise specified, all of the reactions were carried out under an argon atmosphere with magnetic stirring.

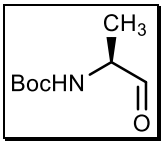
General Experimental Procedure for the Preparation of Amino Aldehydes

To a cold (–78 °C) solution of *tert*-butoxycarbonyl alanine methyl ester (406 mg, 2 mmol) in DCM (20 mL) was added dropwise diisobutylaluminum hydride (1 M in hexane, 4 mL, 4 mmol). The resulting mixture was stirred for 30 min. Then Rochelle salt saturated aqueous solution (50 mL) was added, and the

resulting mixture was stirred at room temperature until two phases were separated. After extraction with dichloromethane (3 × 15 mL), the organic layers were washed with brine, dried ($Na_2SO_4$), and concentrated. The crude material was purified by chromatography (silica gel, hexanes/ethyl acetate (7:3)) to afford the desired compound (288 mg, 83%).

General experimental procedure for preparation of aldehyde from Weinreb amide

To ice bath cold solution of ester (1mmol) in diethyl ether (20mL) was added lithium aluminum hydride (2mmol) dropwise. The mixture stirred for 30 minutes and then it was quenched adding water (5mL) and then extracted with diethyl ether (3 × 15 mL). The organic layers were washed with 1 M hydrochloric acid (15 mL), sodium bicarbonate saturated aqueous solution (15 mL), and brine, dried ($Na_2SO_4$), and concentrated. Aldehyde can be used without further purification.




$^1$H NMR (300 MHz, cdcl$_3$) δ 9.38 (s, 1H), 5.44 (t, *J* = 10.3 Hz, 1H), 4.22 – 3.77 (m, 1H), 1.38 (s, 18H), 1.16 (d, *J* = 1.4 Hz, 3H).

$^{13}$C NMR (75 MHz, cdcl$_3$) δ 200.19, 155.36, 79.68, 55.30, 28.10, 14.37.

General experimental procedure for preparation of nitroaldols

To an ice bath solution of aminoaldehyde (1mmol) in dichloromethane (1mL) was added nitroalkane (6mmol) and triethylamine (0.3mmol). The mixture stirred for 8h, and then it was quenched adding ammonium chloride saturated aqueous solution (25mL) and then extracted with dichloromethane (3 × 15 mL). The organic layers were washed with 1 M hydrochloric acid (15 mL), sodium bicarbonate saturated aqueous solution (15 mL), and brine, dried ($Na_2SO_4$), and concentrated. Nitroaldols can be used without further purification.

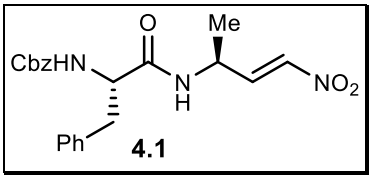
General experimental procedure for preparation of dipeptidyl nitroaldols

To an ice bath solution of nitroaldol (1mmol) in dichloromethane (1mL) was added trifluoroacetic acid (1.5mL) in dichloromethane (1.5mL). The mixture stirred for 30 minutes, and then it was directly concentrated. The resulting salt was dissolved in dichloromethane (10mL) or dimethylformamide (10mL) and stirred in ice bath. Then desired aminoacid was added (1.1mmol) followed by hydroxo benzotriazole (1.1mmol), di*iso*propylamine (4 mmol) and EDC (1.1mmol). It stirred for 8h and then it was quenched adding ammonium chloride saturated aqueous solution (25mL) and then extracted with dichloromethane (3 × 15 mL). The organic layers were washed with 1 M hydrochloric acid (15 mL), sodium bicarbonate saturated aqueous solution (15 mL), and brine, dried ($Na_2SO_4$), and concentrated. Dipeptidylnitroaldols can be used without further purification.

General experimental procedure for preparation of nitroalkenes

To an ice bath solution of nitroaldol (1mmol) in dichloromethane (10mL) was added mesylchloride (2mmol) and slowly di*iso*propylamine (4mmol). The

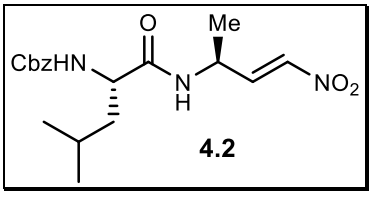
mixture stirred for 2h and then it was quenched adding ammonium chloride saturated aqueous solution (25mL) and then extracted with dichloromethane (3 × 15 mL). The organic layers were washed with 1 M hydrochloric acid (15 mL), sodium bicarbonate saturated aqueous solution (15 mL), and brine, dried ($Na_2SO_4$), and concentrated. The crude material was purified by chromatography (silica gel, hexanes/ethyl acetate (9:1 to 7:3)) to afford the desired compound.



**4.1**

$^1$H NMR (500 MHz, cdcl$_3$) δ 7.41 – 7.23 (m, 8H), 7.16 (d, *J* = 6.6 Hz, 2H), 6.87 (dd, *J* = 13.4, 5.6 Hz, 1H), 6.66 (d, *J* = 13.3 Hz, 1H), 5.68 (s, 1H), 5.06 (s, 2H), 4.40 (s, 1H), 3.05 (ddd, *J* = 21.9, 13.4, 7.5 Hz, 2H), 1.17 (d, *J* = 7.1 Hz, 3H).

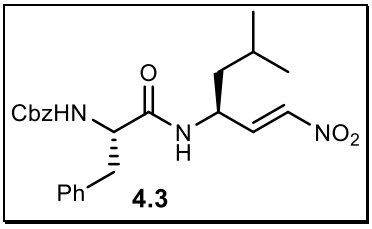
$^{13}$C NMR (126 MHz, cdcl$_3$) δ 170.58, 156.14, 141.55, 139.54, 135.96, 129.22, 129.22, 128.84, 128.84, 128.59, 128.59, 128.34, 127.91, 127.47, 77.30, 77.30, 77.05, 77.05, 76.79, 76.79, 67.25, 56.64, 43.13, 38.71, 19.46.



**4.2**

$^1$H NMR (500 MHz, cdcl$_3$) δ 7.42 – 7.25 (m, 4H), 7.11 (ddd, *J* = 18.9, 5.7, 3.4 Hz, 1H), 5.90 – 5.74 (m, 1H), 5.18 – 4.98 (m, 2H), 4.80 – 4.61 (m, 1H), 4.32 – 4.10 (m, 1H), 1.73 – 1.49 (m, 3H), 1.36 – 1.29 (m, 1H), 1.25 – 1.18 (m, 2H), 0.96 – 0.75 (m, 6H).

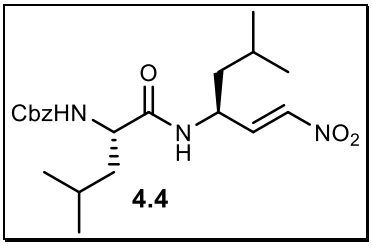
$^{13}$C NMR (126 MHz, cdcl$_3$) δ 172.35, 156.61, 142.29, 142.22, 139.69, 139.62, 136.04, 135.97, 128.55, 128.27, 127.94, 127.86, 77.35, 77.09, 76.84, 67.24, 53.61, 43.35, 41.14, 24.71, 22.81, 21.84, 19.37.




**4.3**

$^{1}$H NMR (500 MHz, cdcl$_3$) δ 7.41 – 7.00 (m, 71H), 6.85 (dd, *J* = 13.4, 6.1 Hz, 5H), 6.69 (d, *J* = 13.4 Hz, 5H), 6.11 (s, 7H), 5.52 (d, *J* = 6.3 Hz, 7H), 5.16 – 5.03 (m, 14H), 4.73 – 4.58 (m, 8H), 4.41 (dt, *J* = 14.3, 7.2 Hz, 8H), 3.20 – 2.94 (m, 16H), 1.52 (dt, *J* = 13.3, 6.6 Hz, 6H), 1.44 – 1.19 (m, 18H), 0.98 – 0.66 (m, 43H).

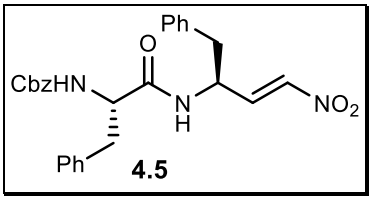
$^{13}$C NMR (126 MHz, cdcl$_3$) δ 170.60, 156.08, 141.01, 139.90, 139.75, 135.93, 129.21, 128.87, 128.84, 128.61, 128.36, 128.06, 127.98, 127.49, 127.25, 77.29, 77.03, 76.78, 67.28, 56.66, 45.78, 42.76, 38.44, 24.59, 22.50, 21.91.




**4.4**

$^{1}$H NMR (500 MHz, cdcl$_3$) δ 7.42 – 7.23 (m, 35H), 7.17 – 7.03 (m, 13H), 6.80 (s, 8H), 5.72 – 5.47 (m, 9H), 5.21 – 4.99 (m, 16H), 4.79 – 4.60 (m, 9H), 4.19 (s, 10H), 1.74 – 1.59 (m, 21H), 1.59 – 1.50 (m, 10H), 1.50 – 1.36 (m, 15H), 1.06 – 0.71 (m, 89H).

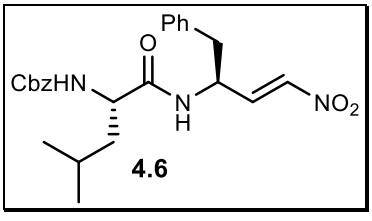
$^{13}$C NMR (126 MHz, cdcl$_3$) δ 172.13, 156.53, 141.64, 139.84, 128.58, 128.56, 128.32, 127.98, 127.91, 127.89, 127.89, 127.88, 77.32, 77.06, 76.81, 67.24, 53.68, 45.94, 42.75, 40.73, 24.74, 22.65, 22.53, 21.93.




**4.5**

$^{1}$H NMR (500 MHz, cdcl$_3$) δ 7.42 – 7.24 (m, 11H), 7.17 (dd, *J* = 18.8, 6.6 Hz, 2H), 6.99 – 6.92 (m, 1H), 6.00 – 5.82 (m, 1H), 5.21 (s, 1H), 5.15 – 5.05 (m, 2H), 4.92 (dd, *J* = 14.1, 7.0 Hz, 1H), 4.37 – 4.25 (m, 1H), 3.15 – 2.92 (m, 2H), 2.89 – 2.65 (m, 2H).

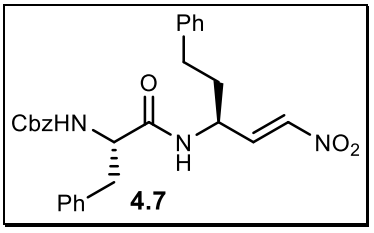
$^{13}$C NMR (75 MHz, cdcl$_3$) δ 170.54, 156.11, 140.39, 140.15, 135.98, 134.74, 129.25, 129.16, 128.94, 128.61, 128.40, 128.17, 128.09, 127.52, 127.44, 77.43, 77.01, 76.58, 67.45, 56.65, 52.37, 48.37, 48.28, 39.79, 37.89.




**4.6**

$^{1}$H NMR (500 MHz, cdcl$_3$) δ 7.40 – 6.86 (m, 21H), 5.81 – 5.68 (m, 1H), 5.64 – 5.50 (m, 1H), 5.18 – 4.88 (m, 5H), 4.26 – 4.17 (m, 1H), 4.08 (ddd, *J* = 8.9, 7.0, 4.4 Hz, 1H), 2.95 – 2.80 (m, 3H), 1.68 – 1.52 (m, 2H), 1.53 – 1.40 (m, 2H), 1.40 – 1.24 (m, 2H), 0.98 – 0.86 (m, 6H), 0.83 (t, *J* = 9.1 Hz, 4H).

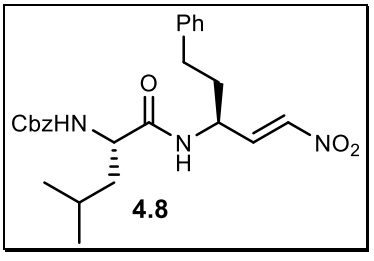
$^{13}$C NMR (126 MHz, cdcl$_3$) δ 172.23, 156.49, 140.67, 140.16, 135.98, 135.46, 129.13, 129.08, 128.81, 128.60, 128.34, 128.28, 127.97, 127.31, 77.39, 77.13, 76.88, 67.27, 53.83, 48.77, 40.68, 40.05, 39.83, 24.69, 22.78, 21.93.




**4.7**

$^{1}$H NMR (500 MHz, cdcl$_3$) δ 7.41 – 7.13 (m, 40H), 7.06 (d, $J$ = 6.5 Hz, 6H), 6.92 – 6.58 (m, 6H), 6.21 (s, 4H), 5.50 (s, 4H), 5.08 (s, 7H), 4.59 (s, 4H), 4.41 (s, 4H), 3.22 – 2.92 (m, 8H), 2.62 – 2.39 (m, 7H), 1.92 – 1.64 (m, 8H).

$^{13}$C NMR (75 MHz, cdcl$_3$) δ 170.78, 156.21, 140.87, 140.56, 140.17, 139.97, 136.09, 135.97, 135.85, 129.24, 128.87, 128.69, 128.60, 128.35, 128.29, 128.03, 127.93, 127.52, 127.37, 126.48, 77.50, 77.07, 76.65, 67.30, 56.76, 47.14, 38.48, 35.27, 35.16, 31.74, 31.66.



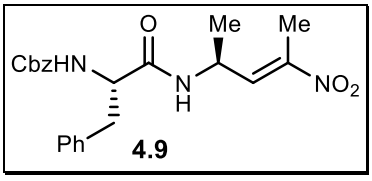

**4.8**

$^{1}$H NMR (500 MHz, cdcl$_3$) δ 7.37 – 7.21 (m, 45H), 7.20 – 6.97 (m, 24H), 6.63 – 6.49 (m, 6H), 5.38 – 5.23 (m, 4H), 5.23 – 5.06 (m, 16H), 4.77 – 4.54 (m, 8H), 4.29 – 3.96 (m, 9H), 2.80 – 2.45 (m, 15H), 2.11 – 1.75 (m, 18H), 1.75 – 1.61 (m, 13H), 1.60 – 1.41 (m, 8H), 1.05 – 0.87 (m, 36H).

$^{13}$C NMR (126 MHz, cdcl$_3$) δ 171.91, 156.60, 140.97, 140.19, 140.15, 140.08, 135.87, 128.76, 128.75, 128.59, 128.29, 128.11, 128.08, 126.56, 67.50, 53.69, 47.34, 40.49, 35.39, 31.85, 24.76, 22.86.

General experimental procedure for preparation of methylated nitroalkenes

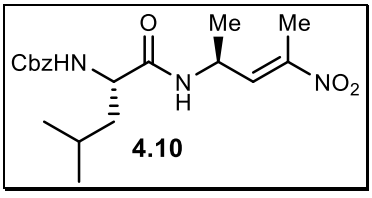
To an ice bath solution of nitroaldol (1mmol) in dichloromethane (10mL) was added mesylchloride (2mmol) and slowly di-*iso*propylamine (4mmol). The mixture stirred for 2h and then it was quenched adding ammonium chloride saturated aqueous solution (25mL) and then extracted with dichloromethane (3 × 15 mL). The organic layers were washed with 1 M hydrochloric acid (15 mL), sodium bicarbonate saturated aqueous solution (15 mL), and brine, dried ($Na_2SO_4$), and concentrated. The crude material was dissolved in dichloromethane (10mL) was added mesylchloride (2mmol) and slowly di-*iso*propylamine (4mmol). The mixture stirred for 2h and then it was quenched adding ammonium chloride saturated aqueous solution (25mL) and then extracted with dichloromethane (3 × 15 mL). The organic layers were washed with 1 M hydrochloric acid (15 mL), sodium bicarbonate saturated aqueous solution (15 mL), and brine, dried ($Na_2SO_4$), and concentrated. The crude material was purified by chromatography (silica gel, hexanes/ethyl acetate (9:1 to 7:3)) to afford the desired compound.




**4.9**

$^{1}$H NMR (500 MHz, cdcl3) δ 7.49 – 7.20 (m, 7H), 7.18 – 7.06 (m, 2H), 6.51 (dd, *J* = 9.7, 1.0 Hz, 1H), 6.28 (s, 1H), 5.63 (d, *J* = 5.9 Hz, 1H), 5.13 – 5.01 (m, 2H), 4.61 (dp, *J* = 9.5, 6.9 Hz, 1H), 4.38 (d, *J* = 6.6 Hz, 1H), 3.11 – 2.90 (m, 2H), 2.23 (dd, *J* = 7.1, 1.0 Hz, 2H), 1.12 (dd, *J* = 19.1, 7.0 Hz, 2H).

$^{13}$C NMR (126 MHz, cdcl3) δ 170.46, 156.06, 148.41, 136.05, 134.82, 129.27, 129.24, 128.70, 128.63, 128.57, 128.28, 127.90, 127.28, 67.15, 56.43, 43.14, 38.94, 19.79, 12.78.




**4.10**

$^{1}$H NMR (300 MHz, cdcl3) δ 7.40 – 7.29 (m, 8H), 6.91 – 6.74 (m, 2H), 6.74 – 6.53 (m, 2H), 5.41 – 5.21 (m, 2H), 5.22 – 4.99 (m, 4H), 4.68 (ddd, *J* = 14.2, 7.1, 2.8 Hz, 2H), 4.26 – 4.00 (m, 2H), 2.32 – 2.14 (m, 5H), 1.74 – 1.40 (m, 6H), 1.40 – 1.11 (m, 7H), 1.03 – 0.81 (m, 13H).

$^{13}$C NMR (75 MHz, cdcl3) δ 171.94, 156.44, 148.61, 135.99, 135.04, 128.56, 128.30, 127.97, 67.24, 53.48, 43.31, 41.08, 40.90, 24.67, 22.88, 21.82, 19.98, 12.79.

General experimental procedure for preparation of cyclopropanes

To an ice bath solution of dimethyl sulfoxide (1 mL), potassium *tert*-butoxide was added (1.05mmol) and stirred for 10 minutes. Alkene (1mmol) was added slowly in dimethyl sulfoxide (1mL) and it stirred for 48h. It was poured onto ice and extracted with diethyl ether. Organic layer was washed with water and brine, dried ($Na_2SO_4$), and concentrated. The crude material was purified by chromatography (silica gel, hexanes/ethyl acetate (9:1 to 7:3)) to afford the desired compound.

General experimental procedure for preparation of nitroepoxides

To cold (–78 °C) solution of nitroalkene (1mmol) in methanol (8mL), hydrogen peroxide (50%, 2 mmol) followed by a solution of sodium hydroxide 2M (3mL) and stirred for 1h. It was poured onto ice and extracted with diethyl ether. Organic layer was washed with water and brine, dried ($Na_2SO_4$), and concentrated. The crude cannot be isolated by silica gel chromatography.

General experimental procedure for the preparation of chlorohydrins

To a stirred solution of chloromethyl phenyl sulfone (2.5 mmol) in tetrahydrofuran (5 mL) at –78 °C was added slowly *n*-buthyl lithium (1.6 M in hexanes, 1.56 mL, 2.5 mmol). The mixture was stirred for 15 min, and then a solution of aldehyde (173 mg, 1 mmol) in tetrahydrofuran (5 mL) was added

slowly. The mixture was stirred for 1.5 h, and the reaction was monitorized by TLC. The reaction was quenched with ammonium chloride saturated aqueous solution (25 mL), and the reaction mixture was allowed to warm to room temperature and then extracted with ethyl ether (3 × 15 mL). The organic layers were washed with 1 M hydrochloric acid (15 mL), sodium bicarbonate saturated aqueous solution (15 mL), and brine, dried ($Na_2SO_4$), and concentrated. The crude material was purified by chromatography (silica gel, hexanes/ethyl acetate (9:1 to 7:3)) to afford the desired compound.

General experimental procedure for the protection of amines

To an ice-bath cooled solution of the amine (1 mmol) in an aqueous solution of 2 M sodium hydroxide (1 mL, 2 mmol) was added the corresponding alkyl chloroformate (1 mmol). The resulting mixture, cooled with the ice-bath, was stirred for 1 h and then was stirred at room temperature for 45 min. The reaction mixture was carefully neutralized using 1 M hydrochloric acid solution and extracted with dichloromethane (3 × 30 mL). The organic layers were washed (brine), dried ($Na_2SO_4$), and concentrated. The crude oil was submitted to the next step without any further purification.

General experimental procedure for addition of Grignard reagents to carbamates

To an ice-bath cooled solution of the carbamate (1 mmol) in tetrahydrofuran (6 mL) was added the corresponding Grignard reagent (6 mmol). The resulting mixture was stirred while being cooled with an ice-bath for 10 min and then stirred at room temperature for 24 h. The reaction was quenched with saturated ammonium chloride solution (10 mL) and extracted with ethyl acetate (3 × 30 mL). The organic layers were washed (brine), dried ($Na_2SO_4$), and

concentrated. The crude oil was purified by silica gel chromatography, eluted with hexanes/ ethyl acetate (7:3), (6:4) and ethyl acetate to afford the corresponding amide.

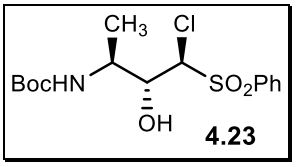
General experimental procedure for one-pot transformation of amines into amides

To an ice-bath cooled solution of the amine (1 mmol) in tetrahydrofuran (6 mL) was added triethylamine (3 mmol) and then the corresponding alkyl chloroformate (1 mmol). The resulting mixture was stirred while being cooled by an ice-bath for 1 h and then stirred at room temperature for 45 min. The reaction mixture was cooled with an ice-bath and the corresponding Grignard reagent (6 mmol) was added. The resulting mixture was stirred while being cooled with the ice-bath for 10 min and then stirred at room temperature for 24 h. The reaction was cautiously quenched with saturated ammonium chloride solution (10 mL) and extracted with ethyl acetate (3 × 30 mL). The organic layers were washed (brine), dried ($Na_2SO_4$), and concentrated. The crude oil was purified by silica gel chromatography, eluted with hexanes/ ethyl acetate (7:3), (6:4) and ethyl acetate to afford the corresponding amide.

General experimental procedure for preparation of chlorhydrins

To cold (–78 °C) stirred solution chloromethylphenyl sulfone (2.5mmol) in tetrahydrofuran (5mL), *n*-buthyl lithium (1.6M in hexanes) (2.5mmol) It stirred for 15 minutes and then a solution of aldehyde (1mmol) in tetrahydrofuran (5mL) was added slowly and stirred for 1.5 hours. It was quenched with ammonium chloride saturated aqueous solution (25mL) and the reaction allowed to warm up to room temperature. Then it was extracted with diethyl ether (3 x 15mL) and the organic layer was washed with hydrochloric acid, sodium bicarbonate saturated

aqueous solution and brine, dried ($Na_2SO_4$), and concentrated. The crude oil was purified by silica gel chromatography, eluted with hexanes/ethyl acetate (9:1 to 6:4) to isolate a first fraction and other purification of this fraction was carried out by silica gel chromatography, eluted with dichloromethane/ ethyl acetate (10:0 to 8:2)




**4.23**

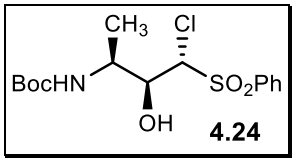
White solid, mp 84–87 °C. Yield 91 mg, 25%. $[\alpha]_D^{20}$ +18.0 ($c$ = 1, $CHCl_3$).

$^1H$ NMR (500 MHz, $CDCl_3$) δ 7.93 (d, $J$ = 7.5 Hz, 1H), 7.67 (t, $J$ = 7.5 Hz, 1H), 7.55 (t, $J$ = 7.8 Hz, 1H), 5.01 (d, $J$ = 9.8 Hz, 1H), 4.68 (d, $J$ = 9.0 Hz, 1H), 4.12 (m, 1H), 3.97 (d, $J$ = 9.3 Hz, 1H), 1.36 (s, 9H), 1.20 (d, $J$ = 6.8 Hz, 2H) ppm.

$^{13}C$ NMR (125 MHz, $CDCl_3$) δ 155.4, 135.3, 134.6, 129.6, 129.2, 79.6, 73.5, 73.4, 47.2, 28.2, 18.4 ppm.

HRMS (ESI) calcd for $C_{15}H_{22}ClNNaO_5S$ ($[M + Na]^+$) 386.0805, found 386.0800.

IR (NaCl) ν 3390, 3020, 2980, 2930, 1711, 1498, 1449, 1393, 1368, 1345, 1322, 1311, 1152, 1100, 1081, 1060, 1018, 998, 822, 699, 686, 578, 553, 543, 456, 432, 418 $cm^{-1}$.




**4.24**

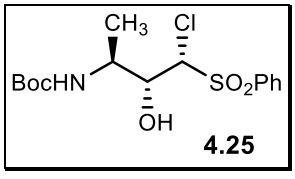
White solid, mp 87–91 °C. Yield 73 mg, 20%. $[\alpha]_D^{20}$ –10.0 (*c* = 1, $CHCl_3$).

$^1$H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, *J* = 7.6 Hz, 1H), 7.76 (d, *J* = 7.3 Hz, 1H), 7.64 (t, *J* = 7.7 Hz, 1H), 5.04 (s, 1H), 4.52 (d, *J* = 9.6 Hz, 1H), 4.24 (d, *J* = 9.5 Hz, 1H), 4.16 (m, 1H), 4.05 (s, 1H), 1.45 (s, 9H), 1.14 (d, *J* = 6.6 Hz, 2H) ppm.

$^{13}$C NMR (125 MHz, $CDCl_3$) δ 155.1, 135.0, 134.6, 130.2, 129.3, 79.6, 73.3, 71.4, 47.6, 28.2, 13.0 ppm.

HRMS (ESI) calcd for $C_{15}H_{22}ClNNaO_5S$ ($[M + Na]^+$) 386.0805, found 386.0805.

IR (NaCl) ν 3444, 3019, 2980, 2360, 2342, 1707, 1500, 1449, 1393, 1368, 1348, 1312, 1220, 1153, 1136, 1099, 1058, 1024, 997, 818, 686, 577, 558, 532, 487, 461, 430, 418 $cm^{-1}$.




**4.25**

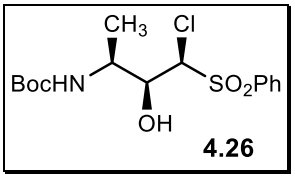
White solid, mp 85–90 °C. Yield 65 mg, 18%. $[\alpha]_D^{20}$ –14.0 (*c* = 1, $CHCl_3$).

$^1$H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.0 Hz, 1H), 7.68 (t, *J* = 7.0 Hz, 1H), 7.57 (t, *J* = 7.6 Hz, 1H), 4.85 (s, 1H), 4.79 (d, *J* = 8.4 Hz, 1H), 4.51 (s, 1H), 3.92 (m, 1H), 3.34 (m, 1H), 1.40 (s, 9H), 1.25 (d, *J* = 6.8 Hz, 2H) ppm.

$^{13}$C NMR (75 MHz, $CDCl_3$) δ 156.8, 136.0, 135.2, 130.7, 129.5, 80.7, 76.9, 71.3, 50.8, 28.9, 18.5 ppm.

HRMS (ESI) calcd for $C_{15}H_{22}ClNNaO_5S$ ($[M + Na]^+$) 386.0805, found 386.0811.

IR (NaCl) ν 3444, 3019, 2980, 2934, 1703, 1504, 1449, 1393, 1368, 1345, 1322, 1311, 1156, 1101, 1083, 1055, 1026, 998, 686, 576, 559, 540, 503, 472, 429, 418 $cm^{-1}$.




**4.26**

White solid, mp 85–89 °C. Yield 44 mg, 12%. $[\alpha]_D^{20}$ +20.0 ($c$ = 1, $CHCl_3$).

$^1H$ NMR (500 MHz, $CDCl_3$) δ 7.97 (d, $J$ = 7.3 Hz, 1H), 7.72 (t, $J$ = 7.5 Hz, 1H), 7.60 (t, $J$ = 7.8 Hz, 1H), 4.95 (s, 1H), 4.58 (d, $J$ = 7.7 Hz, 1H), 4.45 (d, $J$ = 7.6 Hz, 1H), 3.84 (s, 1H), 3.16 (s, 1H), 1.42 (s, 9H), 1.30 (d,$J$ = 6.7 Hz, 2H) ppm.

$^{13}C$ NMR (125 MHz, $CDCl_3$) δ 155.6, 135.5, 134.8, 130.0, 129.2, 80.1, 74.7, 71.5, 49.0, 28.3, 17.5 ppm.

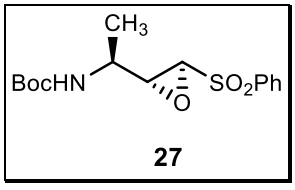
HRMS (ESI) calcd for $C_{15}H_{22}ClNNaO_5S$ ($[M + Na]^+$) 386.0805, found 386.0804.

IR (NaCl) ν 3378, 3019, 2980, 1707, 1504, 1449, 1393, 1368, 1324, 1312, 1154, 1083, 1049, 1028, 686, 587, 576, 540 $cm^{-1}$.

General Experimental Procedure for the Preparation of Epoxysulfones from Chlorohydrins

To an ice-bath-cold solution of chlorohydrin (1 mmol) in dichloromethane/*tert*-butanol (1:1) (3 mL) was added slowly sodium *tert*-butoxide (0.95 mmol) in one portion. The mixture was stirred for 30 min, and then the reaction was quenched with ammonium chloride saturated aqueous solution (25 mL). The resulting mixture was extracted with dichloromethane (3 × 15 mL), and

then the organic layers were washed with 1 M hydrochloric acid (15 mL), sodium bicarbonate saturated aqueous solution (15 mL), and brine, dried ($Na_2SO_4$), and concentrated. The crude material was purified by chromatography (silica gel, hexanes/ethyl acetate (7:3)) to afford the desired compound.




**27**

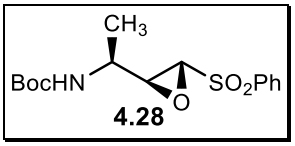
Colorless oil. Yield 275 mg, 84%. $[\alpha]_D^{20}$ +2.0 ($c$ = 1, $CHCl_3$).

$^1$H NMR (500 MHz, $CDCl_3$) δ 7.93 (d, $J$ = 8.3 Hz, 1H), 7.71 (t, $J$ = 6.9 Hz, 1H), 7.60 (t, $J$ = 7.6 Hz, 1H), 4.37 (br s, 1H), 4.17–4.08 (m, 1H), 4.00 (t, $J$ = 1.4 Hz, 1H), 3.69 (d, $J$ = 1.3 Hz, 1H), 1.42 (s, 9H), 1.26 (d, $J$ = 7.0 Hz, 3H) ppm.

$^{13}$C NMR (125 MHz, $CDCl_3$) δ 154.8, 136.0, 134.5, 129.4, 128.8, 80.1, 66.3, 59.9, 44.3, 28.3, 18.4 ppm.

HRMS (ESI) calcd for $C_{15}H_{21}NNaO_5S$ ($[M + Na]^+$) 350.1038, found 350.1032.

IR (NaCl) ν 3020, 2981, 1712, 1498, 1449, 1393, 1368, 1327, 1265, 1083, 1058, 686, 580, 555 $cm^{-1}$.




**4.28**

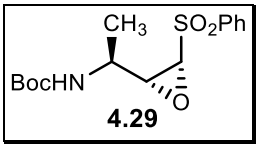
Colorless oil. Yield 258 mg, 79%. $[\alpha]_D^{20}$ +6.0 ($c$ = 1, $CHCl_3$).

$^1H$ NMR (500 MHz, $CDCl_3$) δ 7.94 (d, $J$ = 7.9 Hz, 1H), 7.71 (t, $J$ = 7.2 Hz, 1H), 7.60 (t, $J$ = 7.9 Hz, 1H), 4.54 (m, 1H), 4.23 (m, 1H), 3.68 (m, 1H), 3.57 (dd, $J$ = 6.1, 1.0 Hz, 1H), 1.44 (s, 9H), 1.23 (d, $J$ = 6.9 Hz, 3H) ppm.

$^{13}C$ NMR (125 MHz, $CDCl_3$) δ 154.9, 137.1, 134.5, 129.4, 128.8, 80.2, 67.6, 59.5, 46.4, 28.2, 17.0 ppm.

HRMS (ESI) calcd for $C_{15}H_{21}NNaO_5S$ ($[M + Na]^+$) 350.1038, found 350.1039.

IR (NaCl) ν 3019, 2980, 1748, 1733, 1699, 1684, 1498, 1473, 1457, 1329, 1157, 1087, 686, 418 $cm^{-1}$.




**4.29**

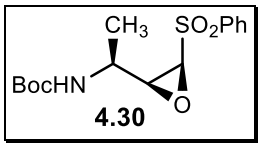
Colorless oil. Yield 229 mg, 70%. $[\alpha]_D^{20}$ –2.0 ($c$ = 1, $CHCl_3$).

$^1H$ NMR (500 MHz, $CDCl_3$) δ 7.93 (d, $J$ = 7.3 Hz, 1H), 7.73 (t, $J$ = 7.5 Hz, 1H), 7.61 (t, $J$ = 7.8 Hz, 1H), 4.55 (s, 1H), 4.15 (m, 1H), 3.87 (m, 1H), 3.70 (m, 1H), 1.43 (s, 9H), 1.28 (d, $J$ = 6.9 Hz, 3H) ppm.

$^{13}C$ NMR (125 MHz, $CDCl_3$) δ 154.6, 136.9, 134.5, 129.5, 128.8, 80.1, 67.5, 59.9, 44.4, 28.3, 18.2 ppm.

HRMS (ESI) calcd for $C_{15}H_{21}NNaO_5S$ ($[M + Na]^+$) 350.1038, found 350.1034.

IR (NaCl) ν 3020, 2981, 1710, 1499, 1456, 1449, 1369, 1329, 1311, 1233, 1017, 583 $cm^{-1}$.




**4.30**

Colorless oil. Yield 209 mg, 64%. $[\alpha]_D^{20}$ –4.0 (*c* = 1, $CHCl_3$).

$^1H$ NMR (500 MHz, $CDCl_3$) δ 7.93 (d, *J* = 7.3 Hz, 1H), 7.71 (t, *J* = 7.5 Hz, 1H), 7.60 (t, *J* = 7.8 Hz, 1H), 4.55 (s, 1H), 4.25 (m, 1H), 3.68 (m, 1H), 3.57 (dd, *J* = 6.1, 1.5 Hz, 1H), 1.44 (s, 9H), 1.23 (d, *J* = 6.9 Hz, 3H) ppm.

$^{13}C$ NMR (125 MHz, $CDCl_3$) δ 154.9, 137.1, 134.4, 129.4, 128.8, 80.2, 67.5, 59.5, 46.4, 28.3, 17.0 ppm.

HRMS (ESI) calcd for $C_{15}H_{21}NNaO_5S$ ($[M + Na]^+$) 350.1038, found 350.1044.

IR (NaCl) ν 3019, 1714, 1497, 1456, 1448, 1369, 1330, 1312, 1157, 1087, 687, 584 $cm^{-1}$.

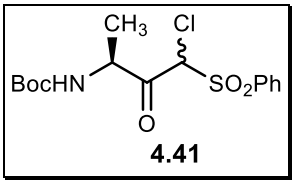
General Experimental Procedure for the Preparation of Chloroketone **4.41** from Chlorohydrin **4.23**

To an ice-bath-cold solution of chlorohydrin **4.1** (363.5 mg, 1 mmol) in dichloromethane (5 mL) was added slowly Dess–Martin periodinane (424.1 mg, 1 mmol) in one portion. The reaction mixture was stirred for 2 h, and then the reaction was quenched with sodium thiosulfate (10%)/sodium carbonate saturated aqueous solution (1:1) (25 mL). The mixture was stirred for 30 min and then extracted with dichloromethane (3 × 15 mL), and the organic layers were washed

with brine, dried ($Na_2SO_4$), and concentrated. The crude material was purified by chromatography (silica gel, hexanes/ethyl acetate (7:3)) to afford the desired compound (yield 321 mg, 89%).

General Experimental Procedure for the Preparation of Chloroketones from Esters

To a stirred solution of chloromethyl phenyl sulfone (3 mmol) in tetrahydrofuran (10 mL) at –78 °C was added slowly *n*-butyl lithium (1.6 M in hexanes, 3 mmol), and the mixture was stirred for 30 min. The amino ester (1 mmol) in tetrahydrofuran (5 mL) was added, and the resulting mixture was gradually warmed to room temperature and stirred overnight. The reaction was quenched with ammonium chloride saturated solution (25 mL). The resulting mixture was extracted with ethyl ether (3 × 15 mL), and then the organic layers were washed with brine, dried ($Na_2SO_4$), and concentrated. The crude material was purified by chromatography (silica gel, hexanes/ethyl acetate (7:3)) to afford the desired compound (yield 217 mg, 60%).




**4.41**

White solid, mp 103–106 °C. $[\alpha]_D^{20}$ –8.0 (*c* = 1, $CHCl_3$).

$^1H$ NMR (300 MHz, $CDCl_3$) δ 7.91 (d, *J* = 7.7 Hz, 4H), 7.74 (t, *J* = 7.5 Hz, 2H), 7.60 (t, *J* = 7.5 Hz, 4H), 5.87 (s, 1H), 5.75 (s, 1H), 5.29 (s, 1H), 4.99 (s, 1H), 4.71 (s, 2H), 1.48 (s, 9H), 1.43 (s, 15H) ppm.

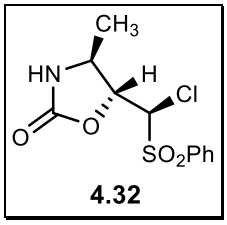
$^{13}$C NMR (75 MHz, $CDCl_3$) δ 196.7, 155.0, 135.3, 135.2, 134.3, 130.6, 130.4, 129.1, 81.0, 80.7, 73.3, 71.9, 55.5, 54.7, 28.3, 28.2, 17.2, 16.1 ppm.

HRMS (ESI) calcd for $C_{15}H_{20}ClNNaO_5S$ ($[M + Na]^+$) 384.0648, found 384.0642.

IR (NaCl) ν 3019, 2981, 2930, 1771, 1540, 1498, 1449, 1394, 1369, 1331, 1313, 1158, 1082, 1072, 1032, 1007, 686, 570, 553, 533, 525, 514, 418 $cm^{-1}$.

General Experimental Procedure for the Preparation of Oxazolidinones

To an ice-bath-cold solution of chlorohydrin (1 mmol) in dichloromethane (3 mL) was added slowly trifluoroacetic acid/ dichloromethane (1:1) (3 mL). The mixture was stirred for 30 min and directly concentrated. Dichloromethane (5 mL) and then phosphate buffer (pH 9) were added, and the mixture was stirred for 15 min and then extracted with dichloromethane. If the aqueous phase still contained free amine as determined by TLC, it was treated with phosphate buffer and 1 M potassium hydroxide. Then the organic layers were washed with brine, dried ($Na_2SO_4$), and concentrated. The crude mixture was dissolved in dichloromethane (6 mL) and cooled with an ice bath. Then a solution of triphosgene (1.2 mmol) in dichloromethane (5 mL) was added dropwise. The resulting mixture was stirred overnight at room temperature. The reaction was quenched with ammonium chloride saturated aqueous solution (25 mL). The resulting mixture was extracted with dichloromethane (3 × 15 mL), and then the organic layers were washed with brine, dried ($Na_2SO_4$), and concentrated. The crude material was purified by chromatography (silica gel, hexanes/ethyl acetate (1:1)) to afford the desired compound.




**4.32**

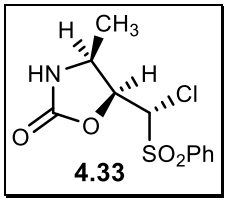
White solid, mp 129–132 °C. Yield 194 mg, 67%. $[\alpha]_D^{20}$ +8.0 (*c* = 1, $CHCl_3$).

$^1H$ NMR (500 MHz, $CDCl_3$) δ 7.98 (d, *J* = 7.6 Hz, 2H), 7.78 (t, *J* = 7.4 Hz, 1H), 7.65 (t, *J* = 7.9 Hz, 2H), 5.90 (br s, 1H), 5.05 (d, *J* = 3.1 Hz, 1H), 5.01 (d, *J* = 2.4 Hz, 1H), 4.35–4.27 (m, 1H), 1.49 (d, *J* = 6.1 Hz, 1H) ppm.

$^{13}C$ NMR (125 MHz, $CDCl_3$) δ 157.1, 135.6, 135.3, 129.7, 129.5, 79.1, 73.9, 49.4, 21.6 ppm.

HRMS (ESI) calcd for $C_{11}H_{12}ClNNaO_4S$ ($[M + Na]^+$) 312.0073, found 312.0074.

IR (NaCl) ν 3019, 2965, 2929, 1698, 1685, 1519, 1508, 1448, 1367, 1328, 1311, 1156, 1085, 686, 590, 571, 418 $cm^{-1}$.




**4.33**

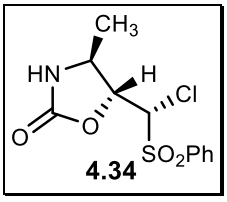
White solid, mp 127–129 °C. Yield 188 mg, 65%. $[\alpha]_D^{20}$ +4.0 (*c* = 1, $CHCl_3$).

$^1H$ NMR (500 MHz, $CDCl_3$) δ 8.05 (d, *J* = 8.5 Hz, 2H), 7.76 (t, *J* = 6.6 Hz, 1H), 7.64 (t, *J* = 7.9 Hz, 2H), 5.43 (br s, 1H), 4.85 (d, *J* = 9.9 Hz, 1H), 4.76 (dd, *J* = 6.6, 9.95 Hz, 1H), 4.01 (m, 1H), 1.32 (d, *J* = 6.4 Hz, 1H) ppm.

$^{13}C$ NMR (125 MHz, $CDCl_3$) δ 157.1, 135.4, 135.3, 130.4, 129.5, 79.6, 70.2, 51.3, 15.8 ppm.

HRMS (ESI) calcd for $C_{11}H_{12}ClNNaO_4S$ ([M + Na]$^+$) 312.0073, found 312.0068.

IR (NaCl) ν 3019, 2960, 2928, 2855, 1698, 1684, 1520, 1448, 1386, 1367, 1328, 1311, 1260, 1156, 1127, 1085, 1051, 1035, 1017, 686, 612, 591, 571, 418 cm$^{-1}$.




**4.34**

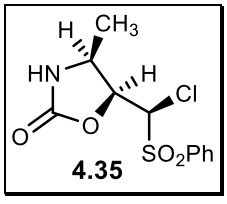
White solid, mp 127–131 °C. Yield 199 mg, 69%. $[\alpha]_D^{20}$ +4.0 (*c* = 1, $CHCl_3$).

$^1$H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.7 Hz, 2H), 7.76 (t, *J* = 7.4 Hz, 1H), 7.63 (t, *J* = 7.6 Hz, 2H), 5.45 (br s, 1H), 4.95 (t, *J* = 3.4 Hz 1H), 4.77 (d, *J* = 2.9 Hz, 1H), 4.04 (m, 1H), 1.44 (d, *J* = 6.2 Hz, 2H) ppm.

$^{13}$C NMR (125 MHz, $CDCl_3$) δ 156.7, 140.9, 135.2, 135.0, 130.6, 129.1, 79.6, 74.7, 51.2, 21.3 ppm.

HRMS (ESI) calcd for $C_{11}H_{12}ClNNaO_4S$ ([M + Na]$^+$) 312.0073, found 312.0077.

IR (NaCl) ν 3019, 2963, 2927, 2871, 1715, 1698, 1540, 1508, 1448, 1386, 1366, 1328, 1311, 1156, 1127, 1085, 1017, 686, 612, 590, 571, 418 cm$^{-1}$.




**4.35**

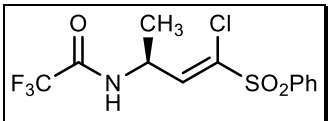
White solid, mp 126–129 °C. Yield 211 mg, 73%. $[\alpha]_D^{20}$ +4.0 (*c* = 1, $CHCl_3$).

$^{1}$H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, *J* = 6.9 Hz, 3.1, 1.5 Hz, 2H), 7.77 (tt, *J* = 7.6, 0.4 Hz, 1H), 7.63 (t, *J* = 8.3 Hz, 2H), 5.43 (dd, *J* = 8.3, 3.2 Hz, 1H), 5.27 (br s, 1H), 4.82 (d, *J* = 3.2 Hz, 1H), 4.33 (dq, *J* = 13.4, 6.8 Hz, 1H), 1.43 (d, *J* = 6.6 Hz, 1H) ppm.

$^{13}$C NMR (75 MHz, $CDCl_3$) δ 157.0, 135.3, 134.5, 130.6, 129.2, 74.6, 72.9, 50.9, 15.3 ppm.

HRMS (ESI) calcd for $C_{11}H_{12}ClNNaO_4S$ ($[M + Na]^+$) 312.0073, found 312.0074.

IR (NaCl) ν 3020, 2956, 2927, 1734, 1457, 1448, 1396, 1378, 1328, 1322, 1312, 1156, 1082, 1056, 1035, 699, 686, 585, 573, 552, 459, 435, 418 $cm^{-1}$.




**4.31**

Yellow oil. Yield 153 mg, 45%. $[\alpha]_D^{20}$ –46.0 (*c* = 1, $CHCl_3$).

$^{1}$H NMR (300 MHz, $CDCl_3$) δ 8.08 (d,*J* = 7.3 Hz, 2H), 7.91 (d, *J* = 7.5 Hz, 2H), 7.69 (m, 3H), 7.59 (m, 3H), 7.18 (d, *J* = 8.1 Hz, 1H), 6.92 (br s, 1H), 6.78 (br s, 1H), 6.32 (d, *J* = 9.2 Hz, 1H), 5.82 (dq, *J* = 9.2, 7.2 Hz, 1H), 4.85 (dq,*J* = 8.1, 6.9 Hz, 1H), 1.53 (d, *J* = 6.9 Hz, 3H), 1.42 (d, *J* = 6.9 Hz, 3H) ppm.

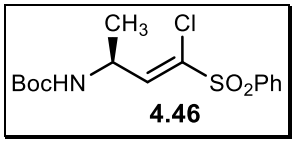
$^{13}$C NMR (75 MHz, $CDCl_3$) δ 157.0 (q, *J* = 37.7 Hz), 142.0, 138.1, 136.3, 134.7, 134.6, 132.4, 129.5, 129.4, 129.0, 115.6 (d, *J* = 289.6 Hz), 45.4, 45.0, 20.5, 18.8 ppm.

HRMS (ESI) calcd for $C_{12}H_{11}ClF_3NNaO_3S$ ($[M + Na]^+$) 363.9998, found 363.9994.

IR (NaCl) ν 3428, 3020, 1728, 1540, 1508, 1448, 1331, 1312, 1288, 1124, 1087, 922, 882, 856, 684, 586, 570, 553, 524, 517, 503, 489, 473, 418 $cm^{-1}$.

General experimental procedure for preparation of chlorovinylsulfones

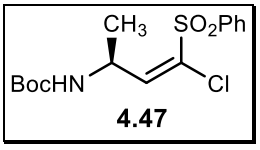
To an ice bath solution of vinylsulfones mixture (1mmol) in dichloromethane (1mL) was added trifluoroacetic acid (1.5mL) in dichloromethane (1.5mL). The mixture stirred for 30 minutes, and then it was directly concentrated. The resulting salt was dissolved in dichloromethane (10mL) or dimethylformamide (10mL) and stirred in ice bath. Then desired aminoacid was added (1.1mmol) followed by hydroxo benzotriazole (1.1mmol), di*iso*propylamine (4 mmol) and EDC (1.1mmol). It stirred for 8h and then it was quenched adding ammonium chloride saturated aqueous solution (25mL) and then extracted with dichloromethane (3 × 15 mL). The organic layers were washed with 1 M hydrochloric acid (15 mL), sodium bicarbonate saturated aqueous solution (15 mL), and brine, dried ($Na_2SO_4$), and concentrated. The crude material was purified by chromatography (silica gel, hexanes/ethyl acetate (1:1)) to afford the desired compound.




**4.46**

$^{1}$H NMR (500 MHz, cdcl$_3$) δ 8.01 – 7.89 (m, 3H), 7.73 – 7.64 (m, 2H), 7.63 – 7.47 (m, 3H), 7.20 – 7.01 (m, 2H), 4.92 – 4.73 (m, 2H), 4.63 – 4.39 (m, 2H), 1.57 – 1.32 (m, 15H), 1.32 – 1.22 (m, 7H).

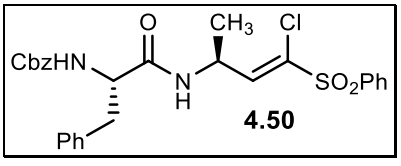
$^{13}$C NMR (126 MHz, cdcl$_3$) δ 154.79, 141.85, 137.03, 134.14, 132.23, 129.20, 128.87, 127.84, 80.02, 45.92, 28.24, 18.95.




**4.47**

$^{1}$H NMR (500 MHz, cdcl3) δ 8.26 – 8.01 (m, 9H), 7.71 – 7.63 (m, 5H), 7.61 – 7.51 (m, 9H), 6.41 – 6.24 (m, 5H), 5.63 – 5.43 (m, 5H), 4.79 – 4.59 (m, 6H), 1.44 (s, 32H), 1.41 (d, *J* = 6.9 Hz, 12H).

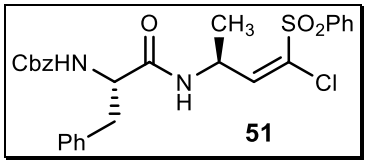
$^{13}$C NMR (126 MHz, cdcl3) δ 154.84, 146.01, 137.92, 134.21, 129.29, 129.09, 128.86, 128.43, 79.91, 45.20, 28.35, 21.15.




**4.50**

$^{1}$H NMR (500 MHz, cdcl3) δ 7.98 – 7.89 (m, 2H), 7.75 – 7.64 (m, 1H), 7.62 – 7.53 (m, 2H), 7.44 – 7.23 (m, 8H), 7.22 – 7.12 (m, 2H), 6.98 – 6.82 (m, 1H), 6.28 – 6.13 (m, 1H), 5.58 – 5.46 (m, 1H), 5.19 – 5.02 (m, 2H), 4.77 – 4.60 (m, 1H), 4.46 – 4.28 (m, 1H), 3.17 – 2.93 (m, 2H), 1.21 – 1.10 (m, 3H).

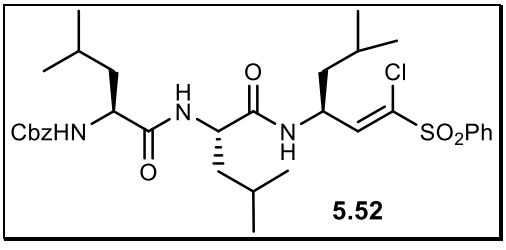
$^{13}$C NMR (126 MHz, cdcl3) δ 170.49, 140.45, 136.84, 136.18, 136.05, 134.24, 132.81, 129.46, 129.28, 129.02, 128.88, 128.57, 128.25, 127.94, 127.28, 77.32, 77.06, 76.81, 67.14, 56.33, 44.65, 41.97, 38.90, 27.00, 24.98, 18.62.




51

$^{1}$H NMR (500 MHz, cdcl$_3$) δ 8.25 – 8.11 (m, 10H), 7.75 – 7.67 (m, 5H), 7.66 – 7.56 (m, 10H), 7.41 – 7.30 (m, 23H), 7.26 – 7.19 (m, 14H), 7.17 – 7.06 (m, 10H), 6.01 – 5.87 (m, 5H), 5.88 – 5.78 (m, 6H), 5.75 – 5.62 (m, 6H), 5.58 – 5.40 (m, 6H), 5.18 – 4.99 (m, 11H), 4.44 – 4.28 (m, 6H), 3.19 – 2.91 (m, 12H), 1.33 – 1.16 (m, 19H).

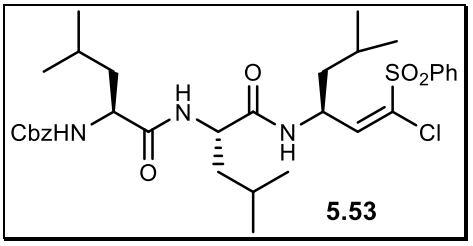
$^{13}$C NMR (126 MHz, cdcl$_3$) δ 169.57, 139.53, 135.93, 135.26, 135.14, 133.33, 131.89, 128.55, 128.36, 128.11, 127.97, 127.66, 127.34, 127.03, 126.37, 76.40, 76.15, 76.15, 75.89, 66.22, 55.41, 43.73, 41.05, 37.99, 26.09, 24.06, 17.71.




5.52

$^{1}$H NMR (500 MHz, cdcl$_3$) δ 7.94 – 7.79 (m, 2H), 7.69 – 7.59 (m, 1H), 7.58 – 7.44 (m, 3H), 7.38 – 7.25 (m, 4H), 7.17 (t, $J$ = 11.7 Hz, 1H), 6.24 (dd, $J$ = 34.5, 6.9 Hz, 1H), 5.19 – 4.99 (m, 2H), 4.92 – 4.77 (m, 1H), 4.50 (d, $J$ = 7.4 Hz, 1H), 4.43 – 4.30 (m, 1H), 1.79 – 1.66 (m, 1H), 1.66 – 1.40 (m, 6H), 1.40 – 1.24 (m, 1H), 0.96 – 0.69 (m, 14H).

$^{13}$C NMR (75 MHz, cdcl$_3$) δ 172.65, 171.31, 156.39, 140.13, 136.94, 136.41, 134.15, 132.90, 129.19, 128.82, 128.45, 127.99, 127.67, 66.78, 53.51, 51.99, 46.68, 42.31, 41.43, 40.65, 24.82, 24.63, 24.57, 22.96, 22.74, 22.62, 22.45, 22.26, 21.95.



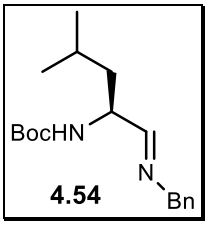

5.53

$^{1}$H NMR (500 MHz, cdcl$_3$) δ 8.20 – 8.08 (m, 5H), 7.72 – 7.63 (m, 3H), 7.63 – 7.52 (m, 6H), 7.46 – 7.31 (m, 13H), 6.70 – 6.58 (m, 3H), 6.41 – 6.24 (m, 6H), 5.84 – 5.62 (m, 4H), 5.19 – 4.99 (m, 9H), 4.47 – 4.32 (m, 4H), 4.21 – 4.04 (m, 4H), 1.88 – 1.76 (m, 3H), 1.74 – 1.61 (m, 16H), 1.61 – 1.44 (m, 12H), 1.05 – 0.72 (m, 54H).

$^{13}$C NMR (75 MHz, cdcl$_3$) δ 172.81, 171.48, 156.56, 140.30, 137.10, 136.57, 134.32, 133.06, 129.36, 128.99, 128.62, 128.16, 127.99, 127.84, 66.94, 53.68, 52.15, 46.84, 42.48, 41.59, 40.82, 24.99, 24.79, 24.74, 23.13, 22.91, 22.79, 22.61, 22.42, 22.12.

General experimental procedure for preparation of imines

To an ice bath solution of amino aldehyde (1mmol) in dichloromethane, benzyilamine was slowly added (2mmol). After stirring 1 hour it was quenched by adding ammonium chloride aqueous saturated solution. It was extracted with dichloromethane (3 x 15mL) washed with brine dried ($MgSO_4$) and concentrated.



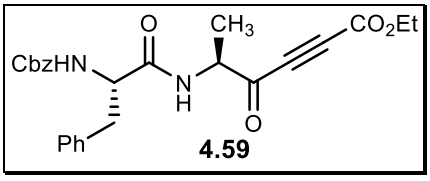

**4.54**

$^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.82 – 7.68 (m, 5H), 7.39 – 7.29 (m, 12H), 7.29 – 7.18 (m, 16H), 5.55 (d, *J* = 6.2 Hz, 4H), 4.70 – 4.54 (m, 10H), 4.35 (s, 4H), 1.78 (td, *J* = 13.3, 6.6 Hz, 5H), 1.66 – 1.54 (m, 6H), 1.48 (d, *J* = 19.2 Hz, 54H), 0.94 (dt, *J* = 12.6, 6.0 Hz, 34H).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 164.79, 155.69, 138.98, 130.80, 128.72, 128.46, 127.79, 126.95, 79.02, 64.07, 52.08, 42.12, 28.39, 23.13.

General experimental procedure for preparation of acetylene

To a cold (-78 °C) solution of acetylene (2.5mmol) in tetrahydrofuran (5mL), *n*-butyllithium (1.6M in hexanes, 2.5mmol) was slowly added. After stirred 30 minutes, ester of aminoacid (1mmol) was added in tetrahydrofuran (5mL). It stirred for overnight and quenched adding ammonium chloride aqueous saturated solution. It was extracted with diethyl ether (3 x 15mL), washed with brine dried ($MgSO_4$) and concentrated.

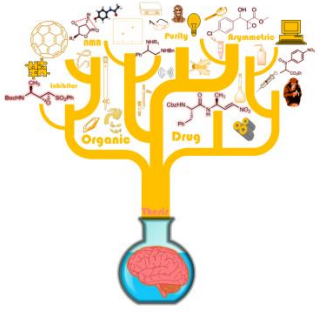



**4.59**

$^{1}$H NMR (500 MHz, cdcl$_3$) δ 7.43 – 7.06 (m, 33H), 6.75 – 6.61 (m, 3H), 5.67 – 5.49 (m, 4H), 5.15 – 4.96 (m, 7H), 4.60 – 4.41 (m, 7H), 4.24 – 4.06 (m, 8H), 3.15 – 2.97 (m, 7H), 1.35 (dd, $J$ = 25.1, 7.2 Hz, 9H), 1.29 – 1.15 (m, 13H).

$^{13}$C NMR (126 MHz, cdcl$_3$) δ 172.41, 170.59, 155.96, 136.38, 136.24, 129.36, 128.53, 128.00, 127.90, 126.95, 66.96, 61.44, 56.02, 48.22, 38.60, 18.23, 14.12.

# General conclusions and future work



English version:

In this thesis, according to the initial objectives, several conclusions can be accomplished:

Cysteine protease inhibitors

- New families of inhibitors have been published against proteasome and parasitic enzymes.
- Epoxysulfones and chloroketones proved to be very good inhibitors of cathepsins H, S and C according to ABPP.
- Nitroalkenes are a new class of cysteine protease reversible inhibitors and a source of new families of inhibitors.
- New families of inhibitors were developed and are waiting to be tested and published.
- A lot of inhibitors have been tested against proteasome, as a part of my formation and opening a new target of inhibition using ABPP.

Synthesis studies

- New route to obtain amides was developed using carbamates as intermediates in a single one pot reaction.
- A new 1,4 sulfur – oxygen interaction was confirmed in a naphtoquinones structures and controlling the high tendency of diastereoisomeric excess when the interaction is present. Furthermore, the understanding of the role of this interaction in the biochemical vitamin K process in the blood coagulation has been achieved.
- A complete study of stereoselectivity of nucleophilic epoxidation of MBH adducts was done, been the *syn* isomer the major in all cases.

- The mechanism of dehydrosulfenylation of 2-aryl esters for furnishing enoates has been determined to be a homolytic process. This mechanism was confirmed by ESI tandem MS radical intermediate monitorization and trapping the radical with tempo.

Future work

- Continue developing the big families of nitrocompounds with all the derivatizations initiated.
- Synthetize new epoxysulfones and chloroketones with the correct structures to be tested against proteasome and other enzymes
- Finish the new aziridines sulfones to check the inhibition power for this warhead.
- Look for new S-O interactions and their effect on new developed compounds.
- Apply the BHM adducts and their epoxidation to develop new compounds and studies.
- Improve the radical intermediates detection to develop new utilities such as mechanism routes and other useful information.
- Complete and evaluate the new interesting families of cysteine proteases synthetized and publish the results.
- Finish the studies of asymmetric synthesis initiated and publish the results.

Versión Española:

En esta tesis, atendiendo a los objetivos iniciales, se pueden extraer diversas conclusiones:

Inhibidores de cisteína protease:

- Nuevas familias de inhibidores han sido publicadas contra proteasoma y otras enzimas.
- Epoxisulfonas y clorocetonas derivadas de la alanina and demostrado ser buenos inhibidores contra cathepsinas H, S y C a tenor de los resultados de ABPP.
- Nitroalquenos han sido desarrollados como nuevos inhibidores reversibles de cisteína proteasa y como una fuente de desarrollo de nuevas familias gracias a sus posibles derivatizaciones.
- Nuevas familias de inhibidores y compuestos han sido desarrolladas durante esta tesis y están pendientes de ser testadas y publicadas
- Muchos compuestos han sido testados contra proteasoma, como parte de la formación de esta tesis doctoral abriendo la puerta a nuevos objetivos de inhibición usando la metodología ABPP.

Estudios de síntesis:

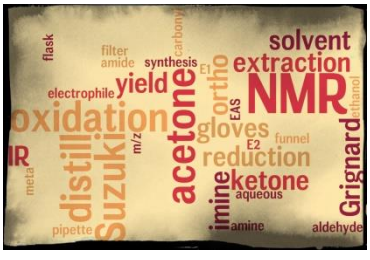
- Una nueva ruta para la obtención de amidas a través de carbamatos ha sido desarrollada utilizando un simple proceso de un paso.
- Una nueva interacción 1,4 azufre-oxígeno en unas naftoquinonas ha sido detectada además de estudiarse la alta tendencia en el exceso diastereoisomérico cuando esta interacción está presente. Esta interacción ayudará a entender mejor su papel en el proceso bioquímico de la vitamina K en la coagulación de la sangre.

- Un completo estudio de la estereoselectividad de la epoxidación nucleófilica de aductos de MBH se llevó a cabo, resultando en todos los casos el isómero *syn* como mayoritario.
- El determinó el mecanismo de deshidrosulfenilación de los 2-aril ésteres como homolítico. Esto se confirmó detectando el radical intermedio con la técnica de ESI tándem MS y atrapando el radical con el tempo.

Trabajo futuro:

- Continuar el desarrollo de las familias de nitro compuestos con todas las derivatizaciones posibles que hay iniciadas.
- Sintetizar nuevas epoxisulfonas y clorocetonas con la correcta estructura para ser testadas de nuevo contra proteasoma y otras enzymas y poder saber todo su potencial.
- Acabar al síntesis de las sulfonil aziridinas para comprobar su potencial y poder saber el futuro de este nuevo tipo de inhibidor.
- Buscar nuevas interacciones S-O y averiguar su efecto sobre posibles reacciones.
- Aplicar la metodología de síntesis y epoxidación de aductos MBH para generar nuevas familias de compuestos y explorar su utilidad.
- Mejorar y ampliar la detección de radicales intermedios de reacción y desarrollar su utilidad para saber rutas, mecanismo u otra información de utilidad.
- Acabar y evaluar los prometedores nuevos inhibidores de cisteína proteasas sintetizados y publicar los resultados.
- Acabar los estudios empezados de síntesis asimétrica y publicar los resultados.

# Selected Spectra



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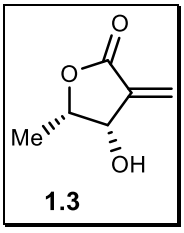
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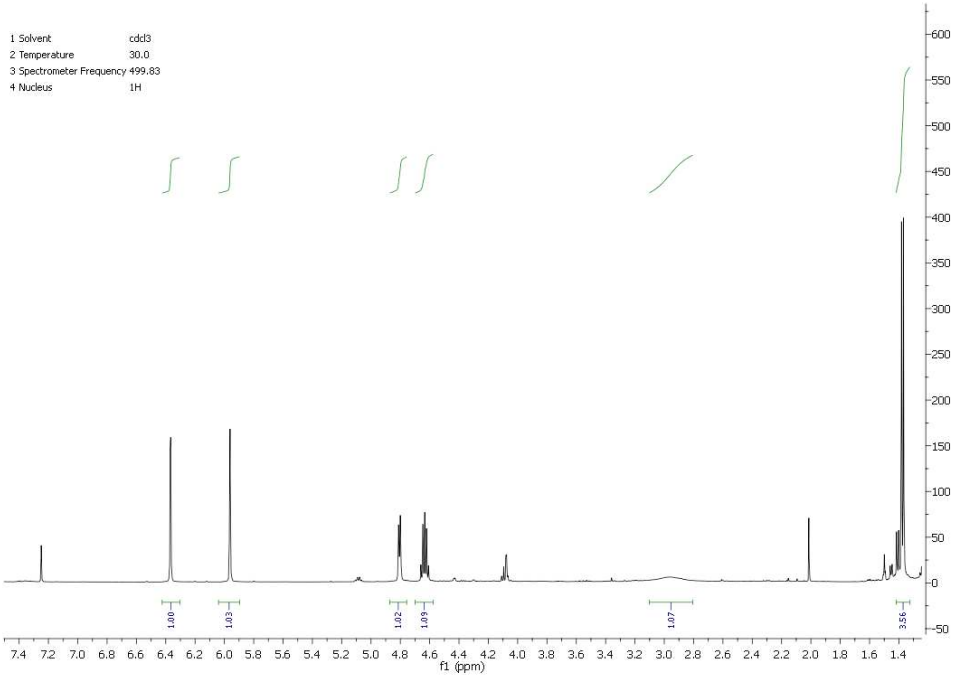
O
Me
OH

1.3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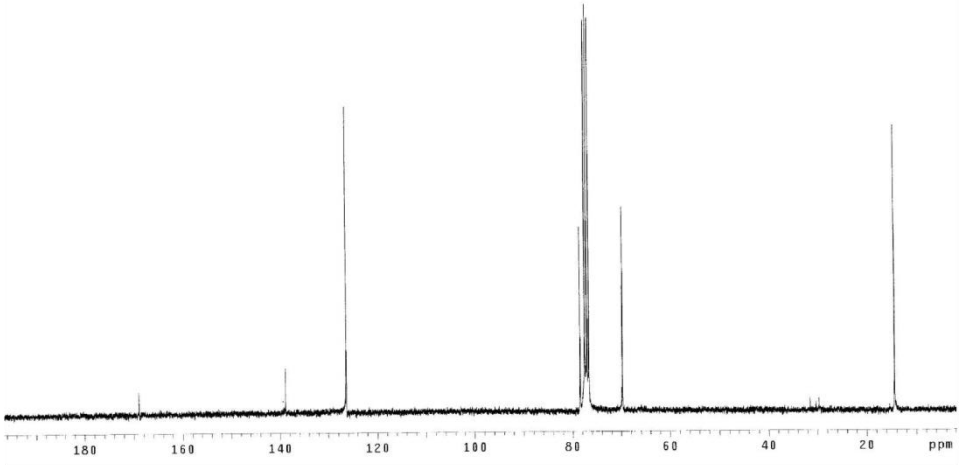
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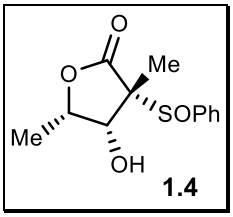
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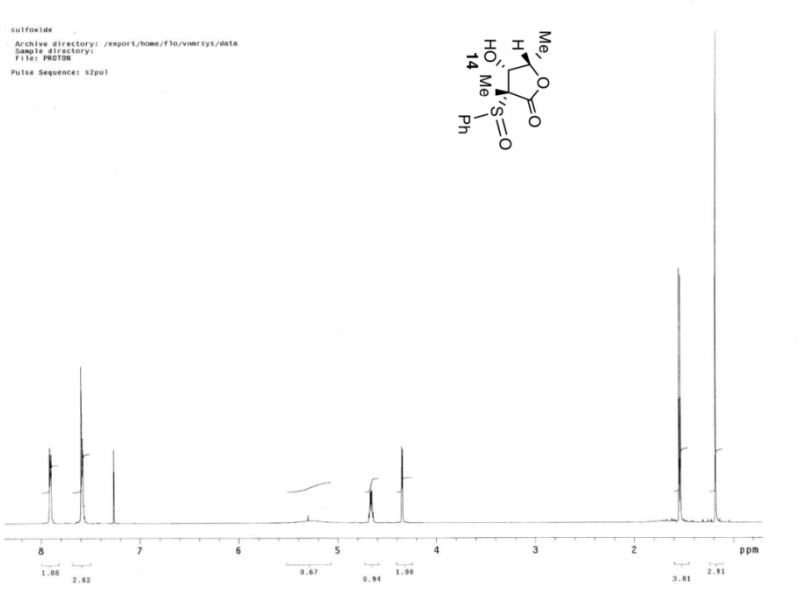
O
Me
SOPh
Me
OH
1.4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Sample directory:
File: PROTON
Pulse Sequence: s2pul
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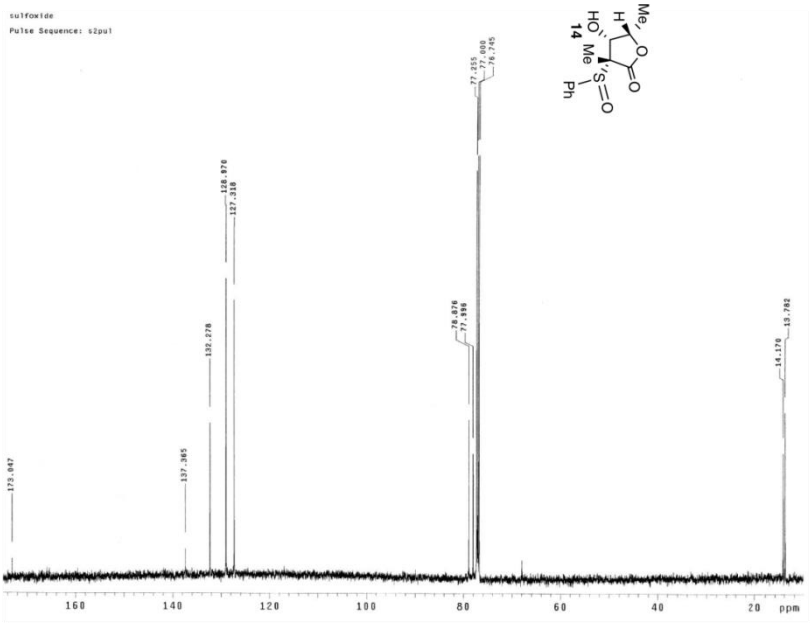
ppm


sulfoxide
Pulse Sequence: s2pul
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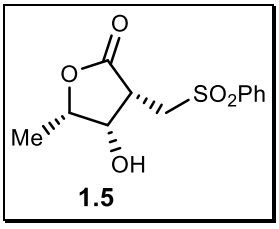
ppm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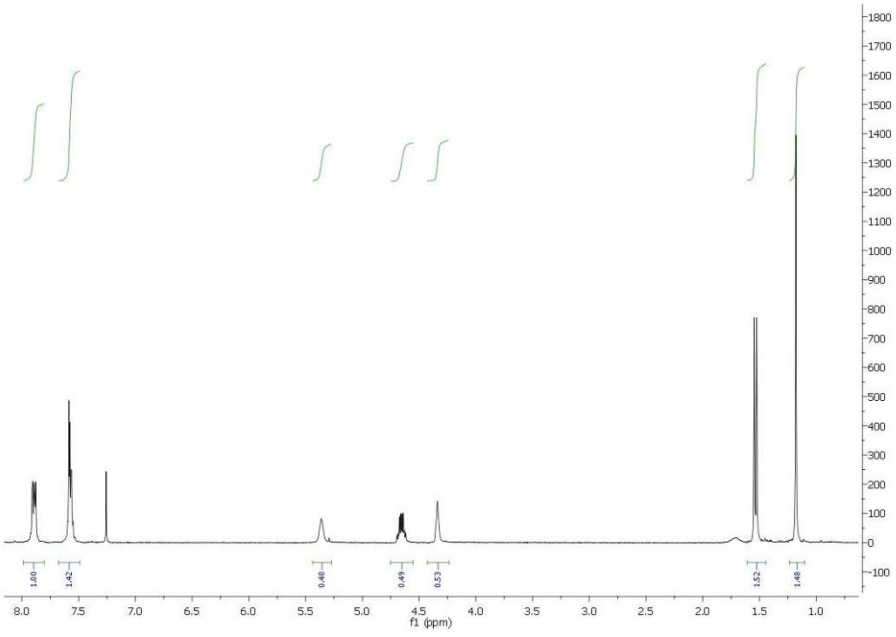
O
SO2Ph
Me
OH

**1.5**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0.53
1.52
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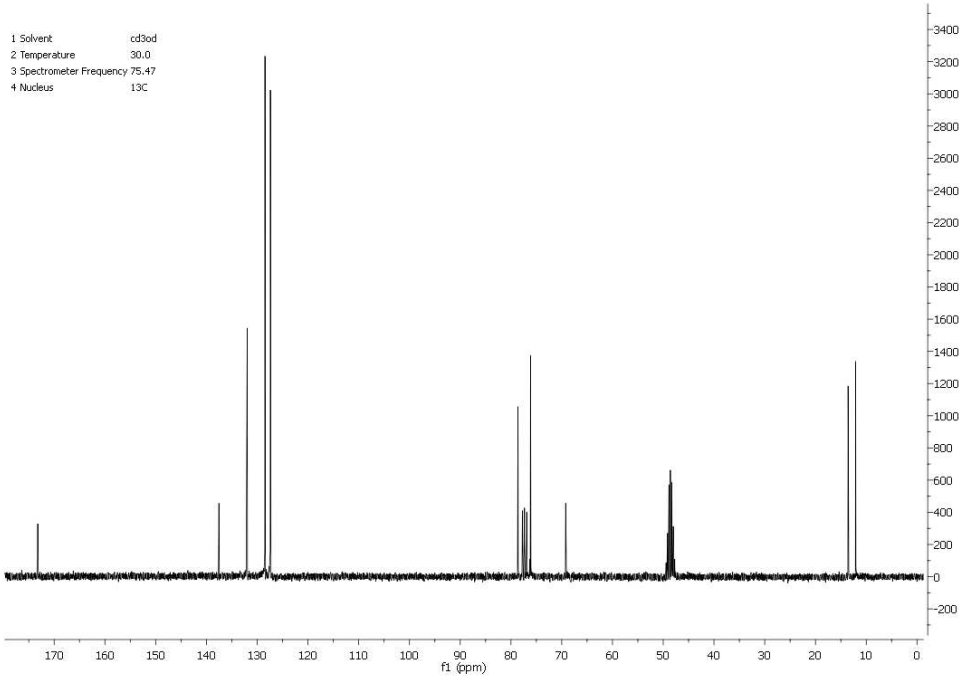
f1 (ppm)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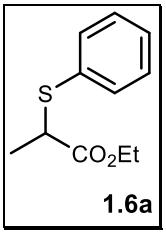
3 Spectrometer Frequency 75.47
4 Nucleus 13C
f1 (ppm)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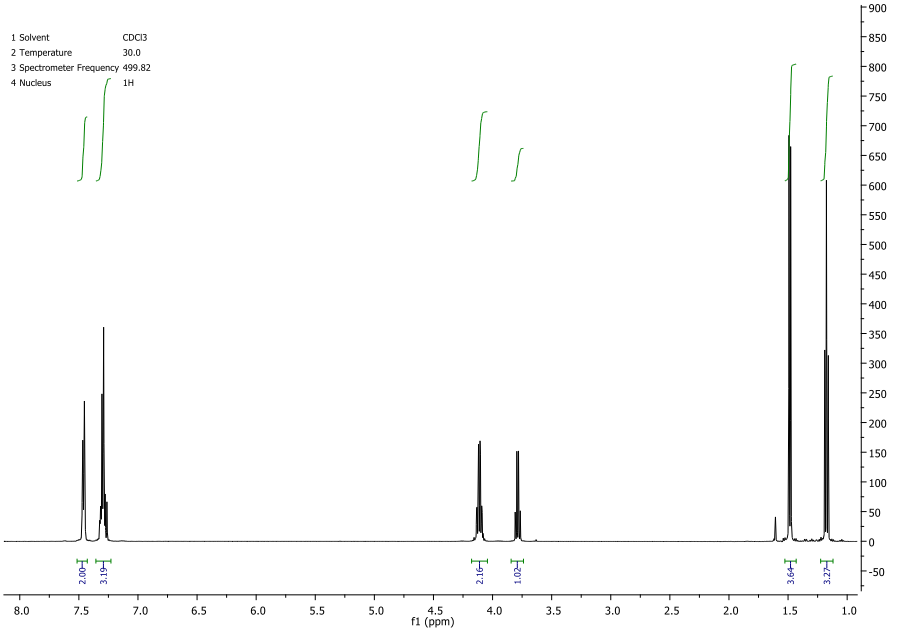
CO2Et

**1.6a**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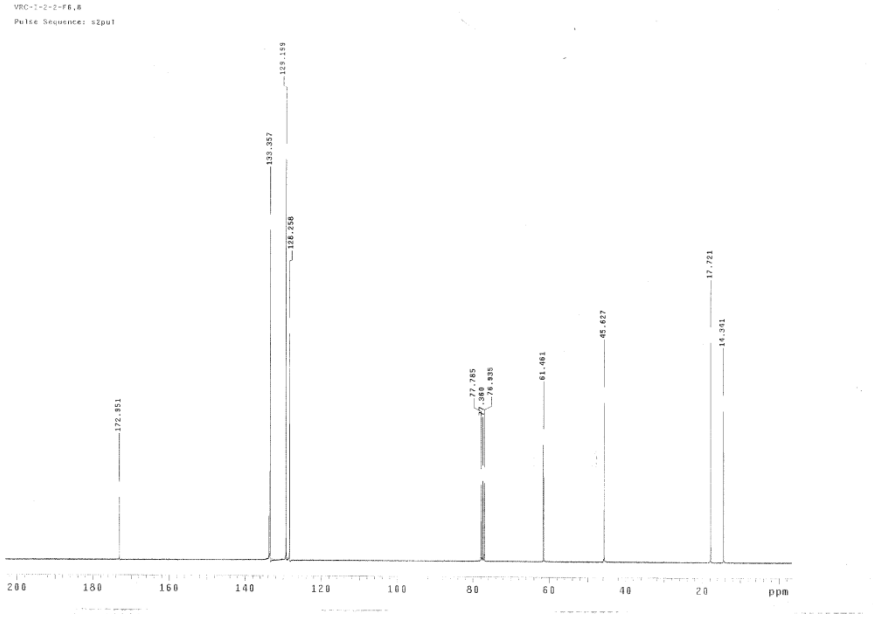
f1 (ppm)


VRC-1-2-2-F6.8
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76.935
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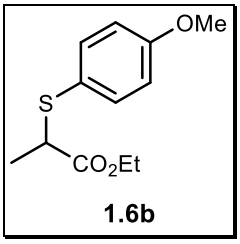
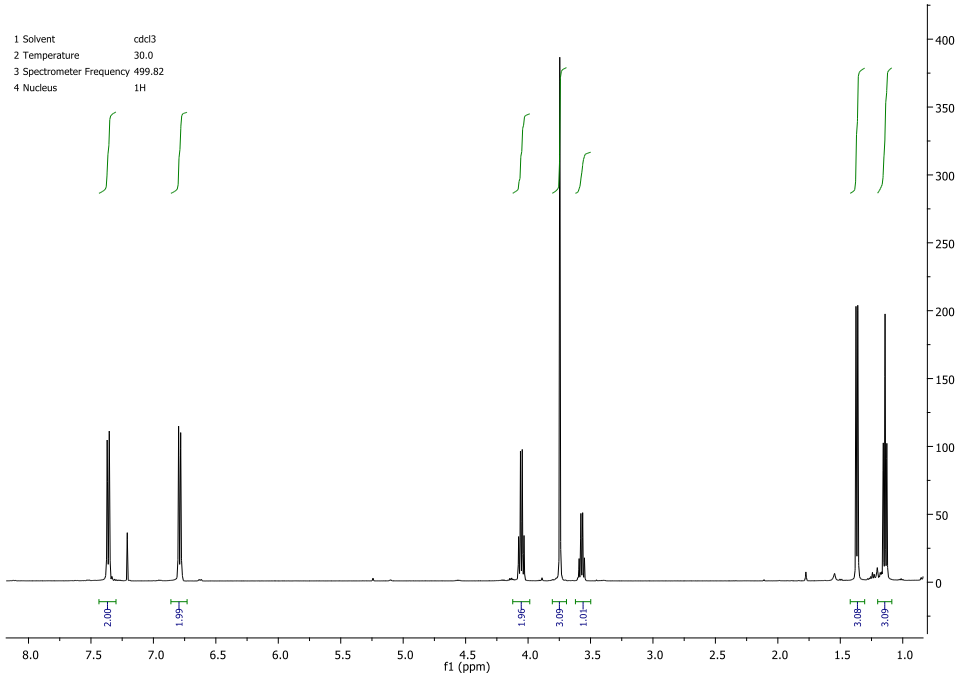
45.627
17.721
14.341
ppm


OMe
S
CO2Et

**1.6b**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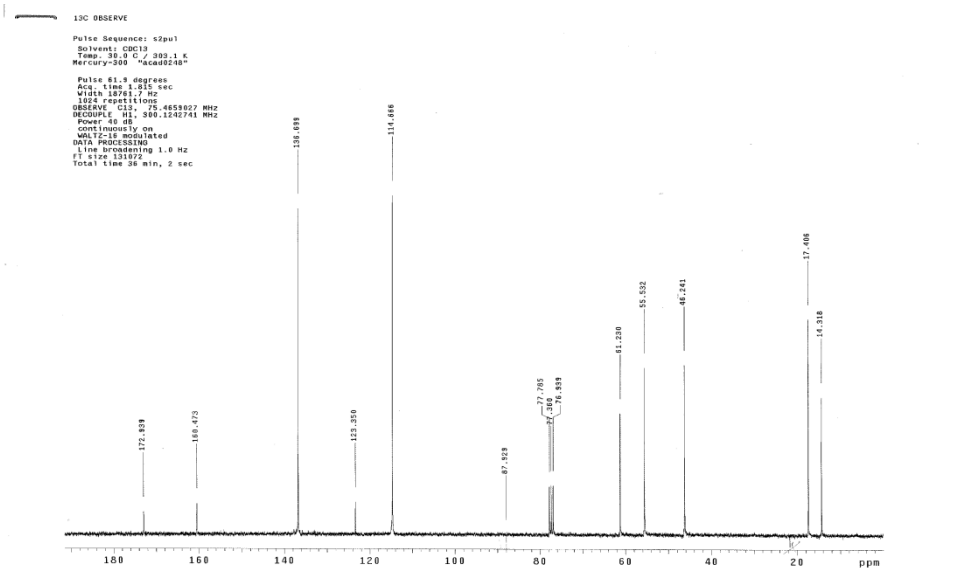
3.09
1.01
3.08
3.09
f1 (ppm)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Solvent: CDCl3
Temp. 30.0 C / 303.1 K
Mercury-300 "acad0248"
Pulse 61.9 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
1024 repetitions
OBSERVE C13, 75.4653027 MHz
DECOUPLE H1, 300.1242741 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 36 min, 2 sec
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160.473
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123.350
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87.528
77.705
77.380
76.989
61.230
55.532
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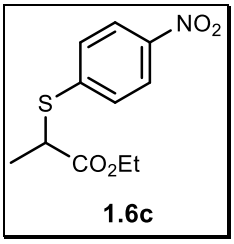
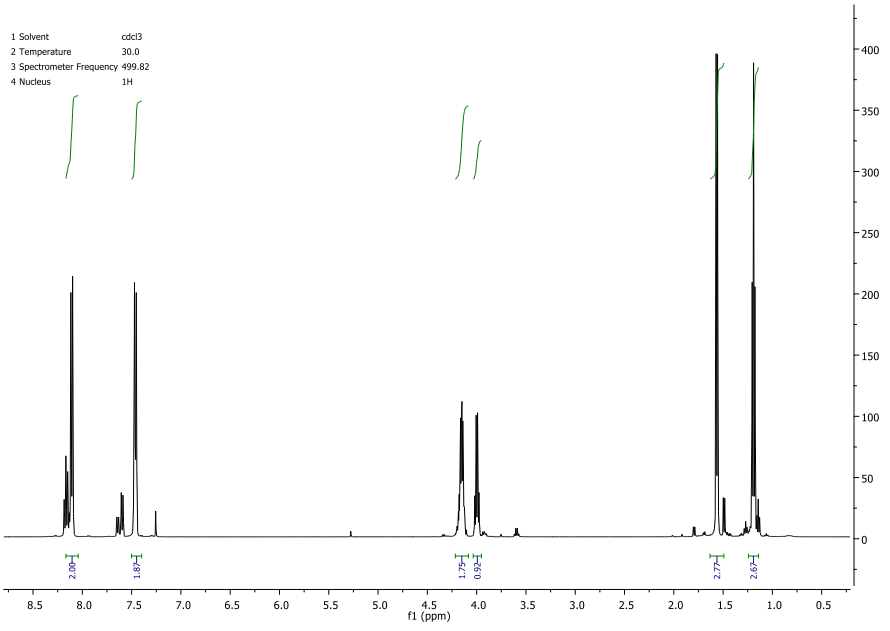
17.406
14.318
ppm


NO2
S
CO2Et

**1.6c**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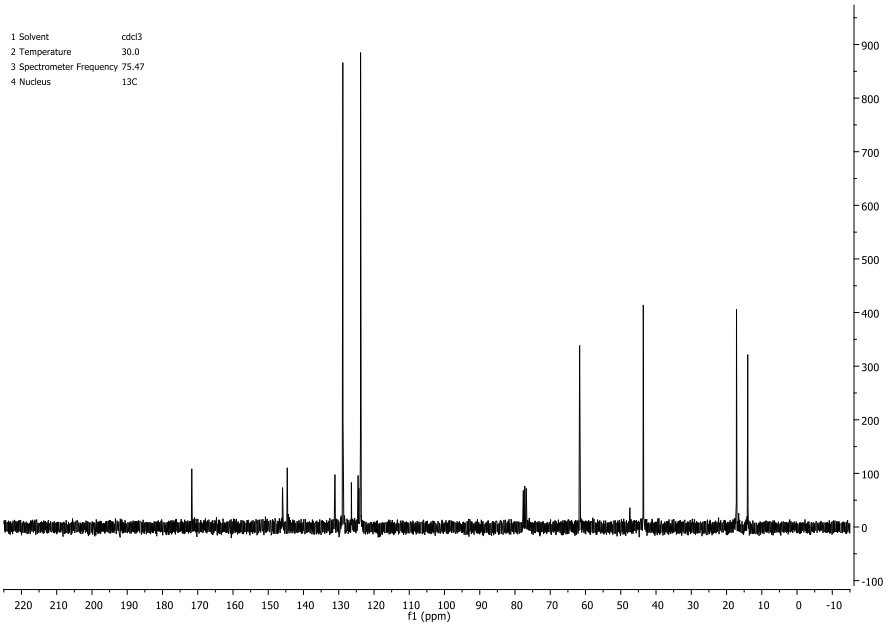
f1 (ppm)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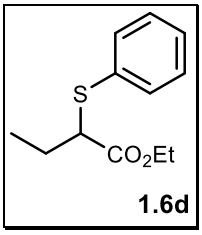
f1 (ppm)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CO2Et
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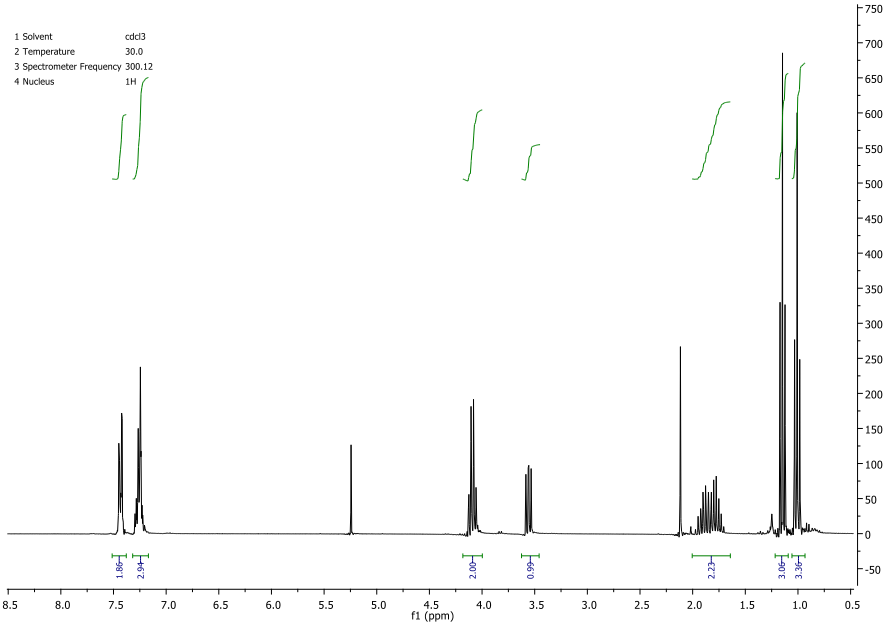
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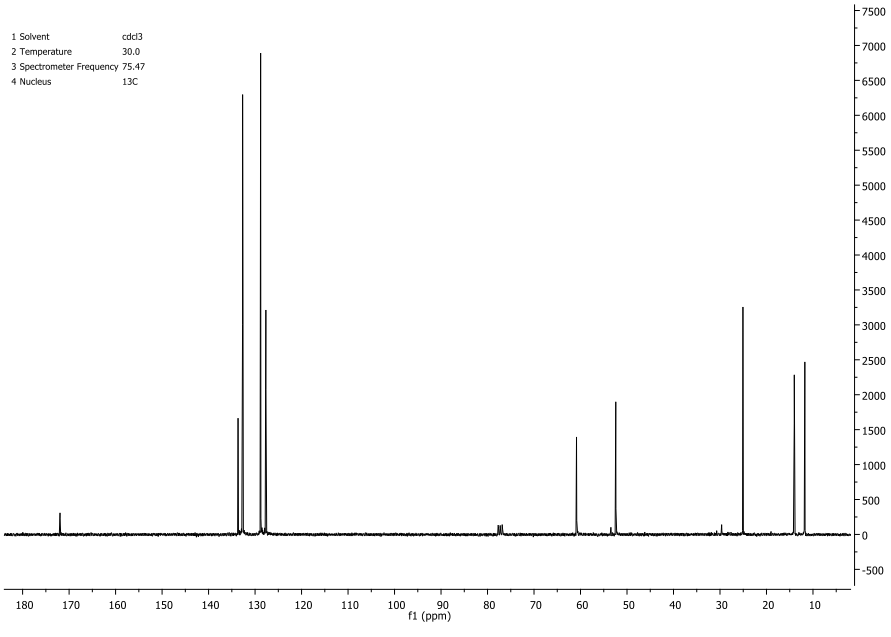
f1 (ppm)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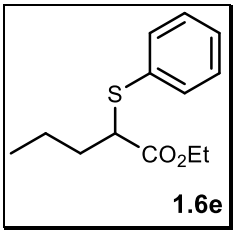
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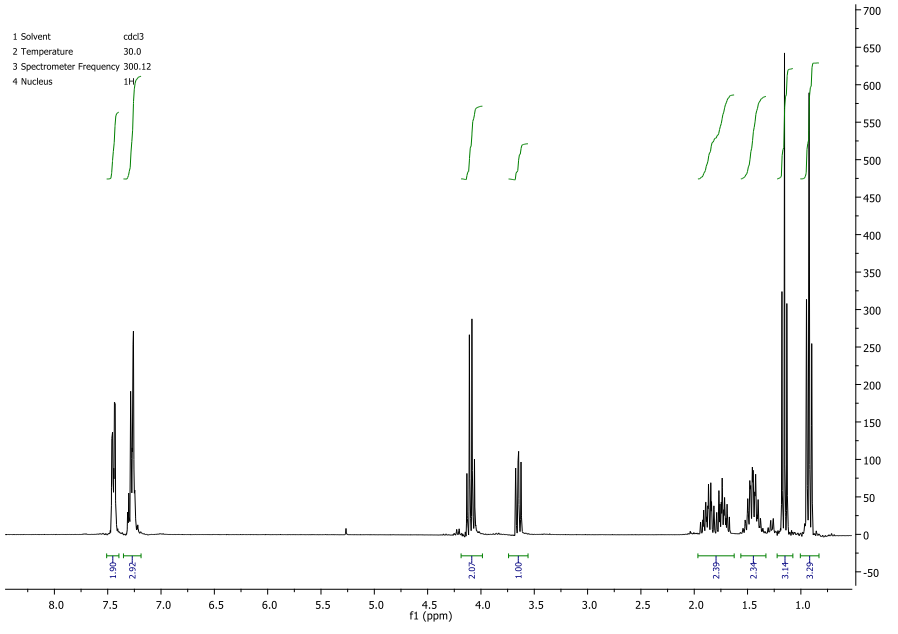
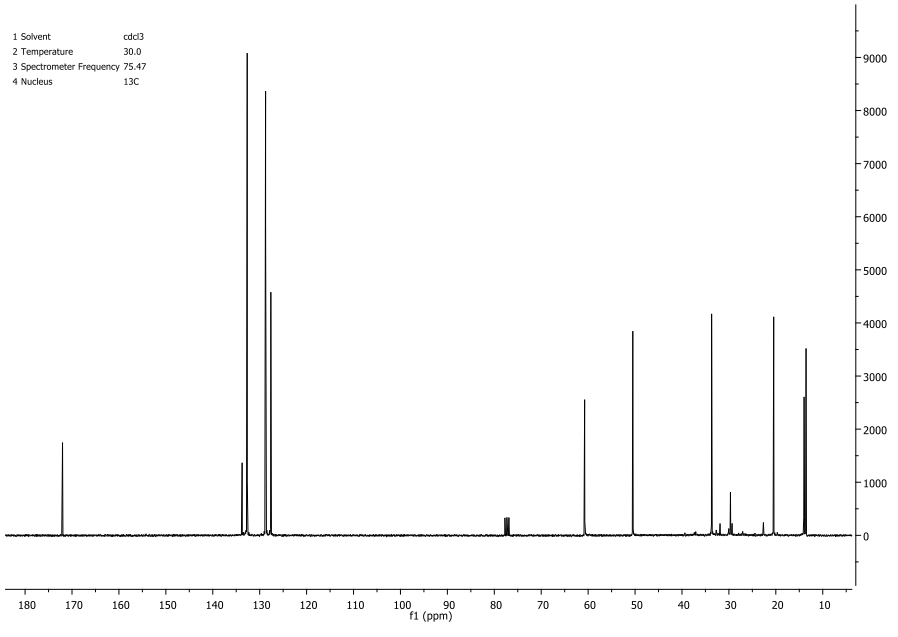
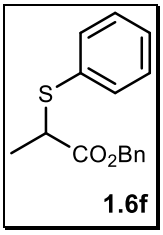
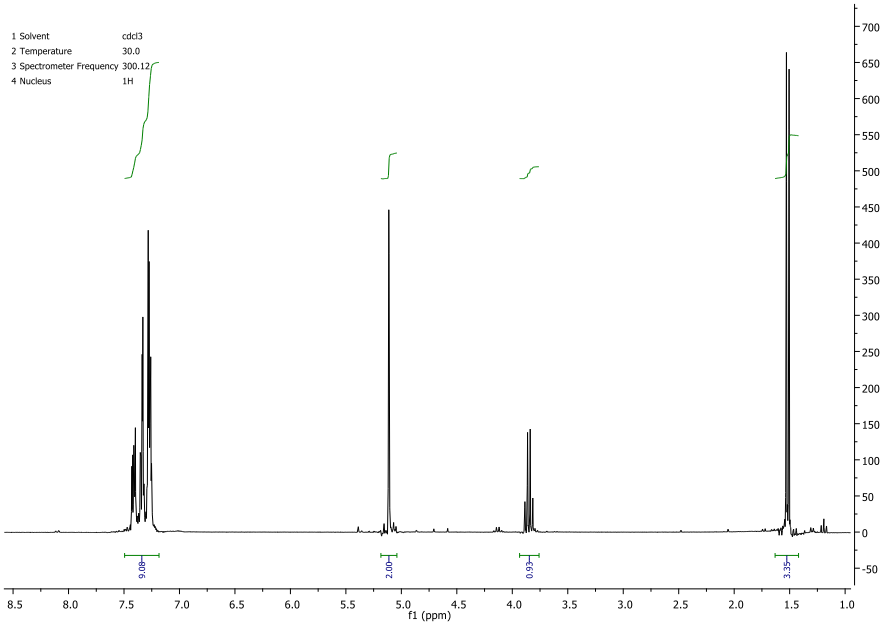
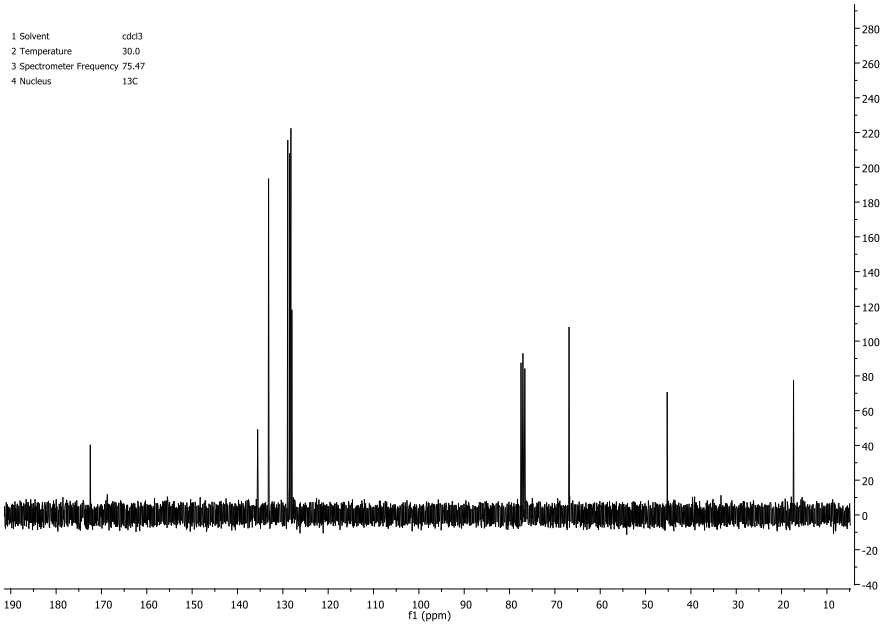
CO2Et
1.6e

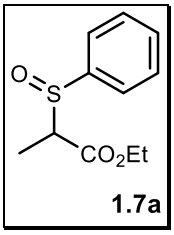

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 300.12
4 Nucleus 1H
1.90
2.93
2.07
1.00
2.39
2.34
3.14
3.29
f1 (ppm)


1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 75.47
4 Nucleus 13C
f1 (ppm)

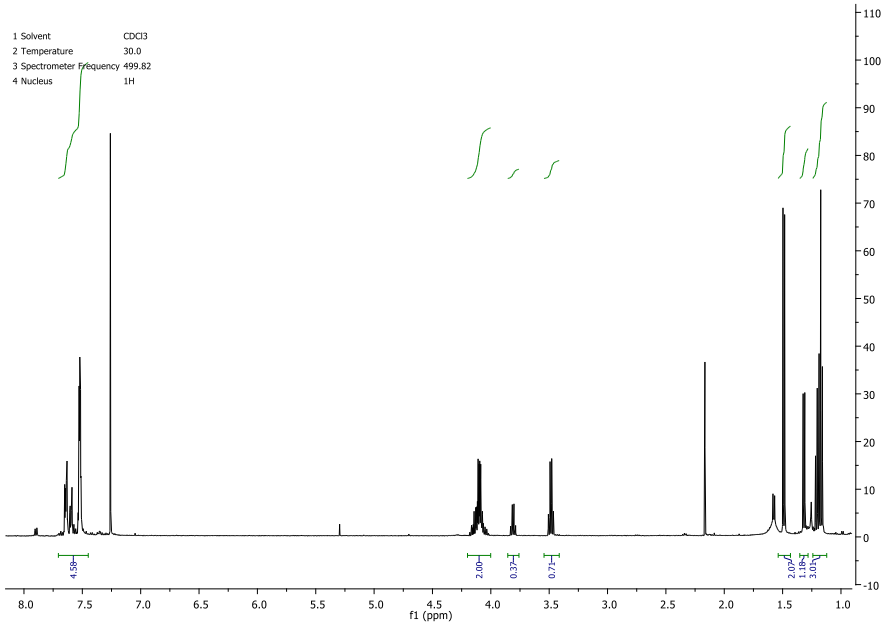

S
CO2Bn
1.6f


1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 300.12
4 Nucleus 1H
9.08
2.00
0.93
3.35
f1 (ppm)

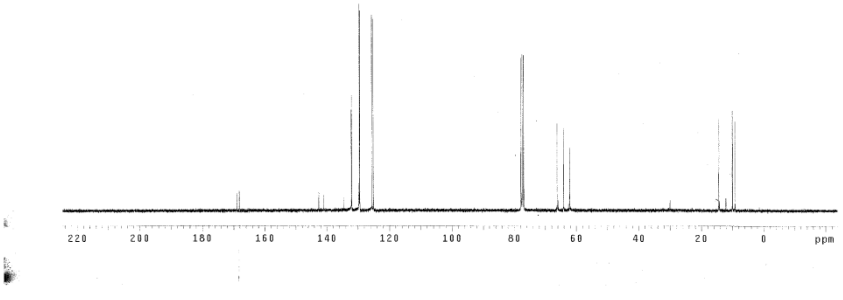

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 75.47
4 Nucleus 13C
f1 (ppm)

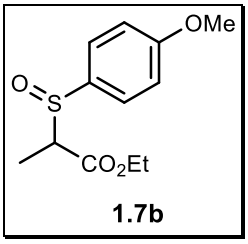

O
S
CO2Et

**1.7a**

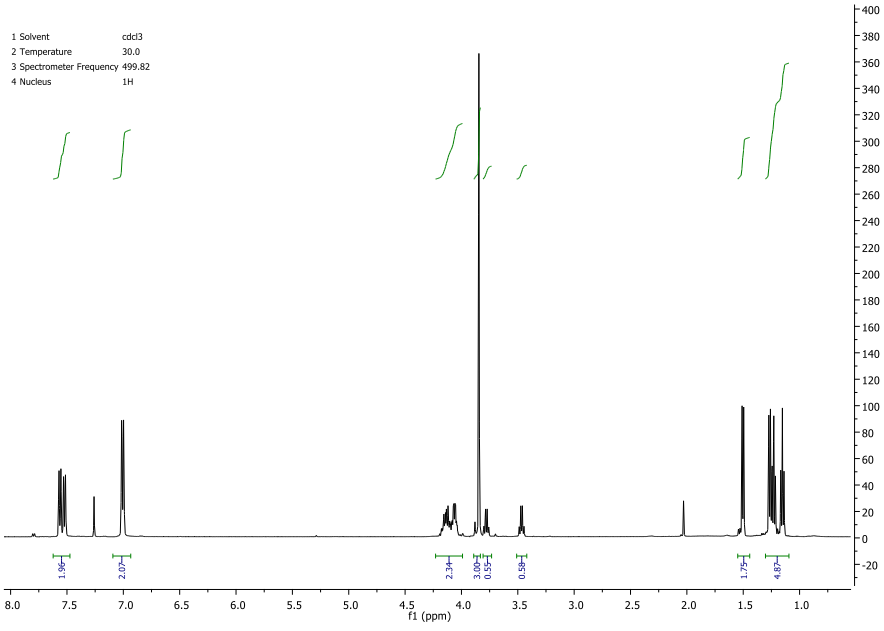

1 Solvent CDCl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
4.59
2.00
0.37
0.71
2.07
1.18
3.01
f1 (ppm)

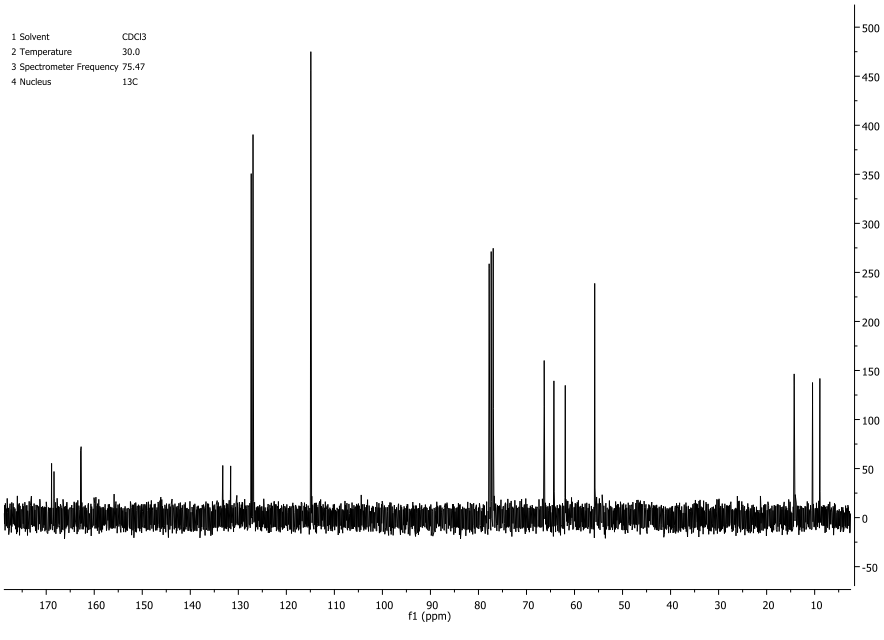

VRC-I-3-1_2C13
Pulse Sequence: s2pul

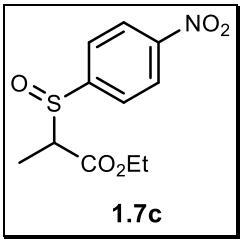

ppm


OMe
O
S
CO2Et

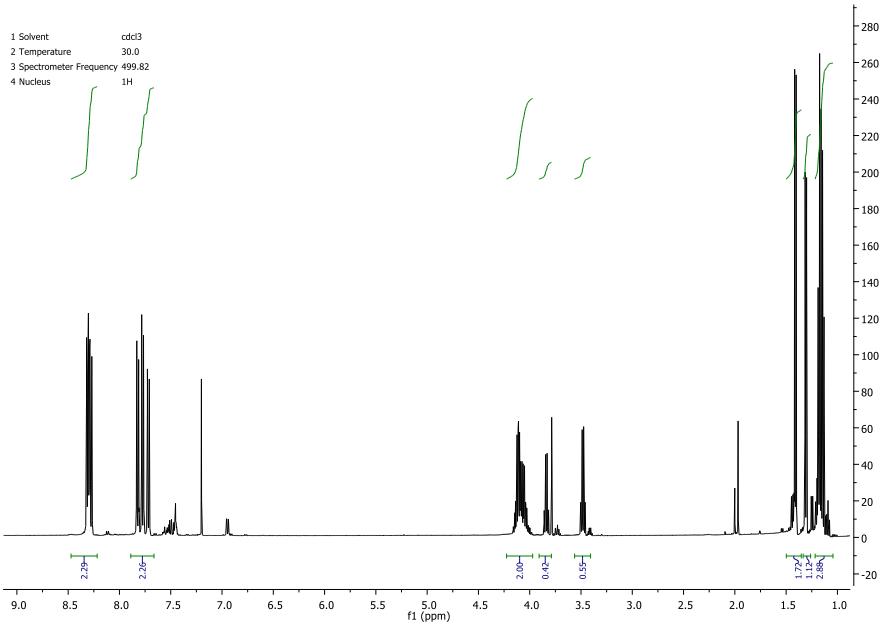
**1.7b**

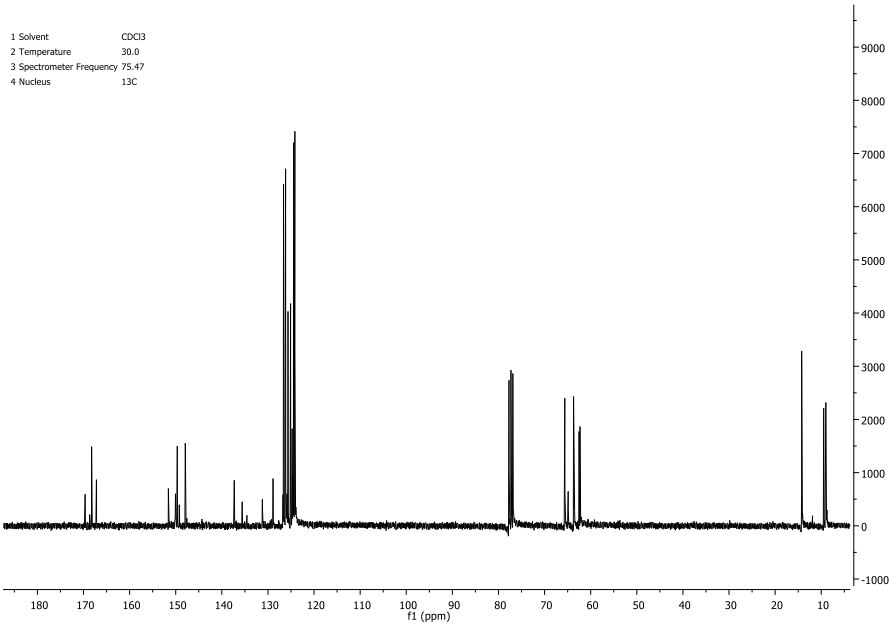

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
1.96
2.07
2.34
3.00
0.55
0.58
1.75
4.87
f1 (ppm)

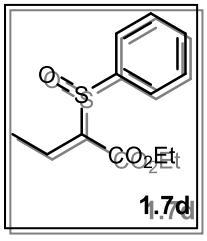

1 Solvent CDCl3
2 Temperature 30.0
3 Spectrometer Frequency 75.47
4 Nucleus 13C
f1 (ppm)

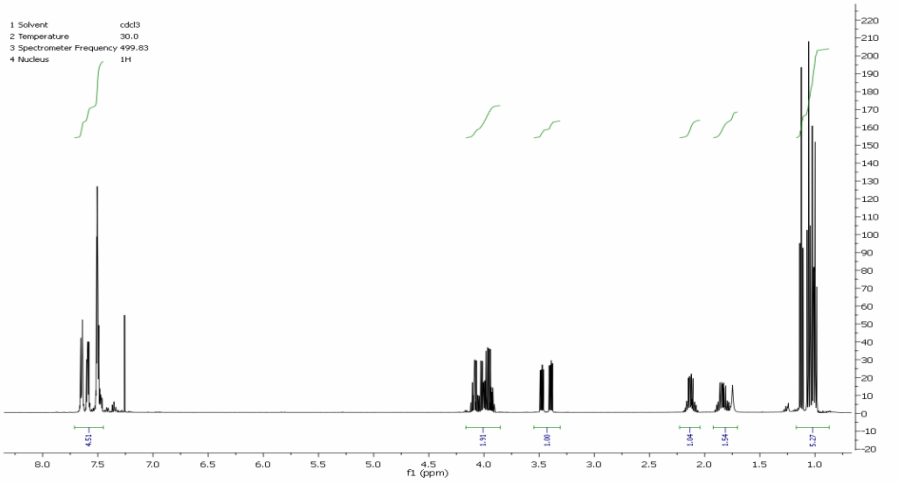

NO2
O
S
CO2Et

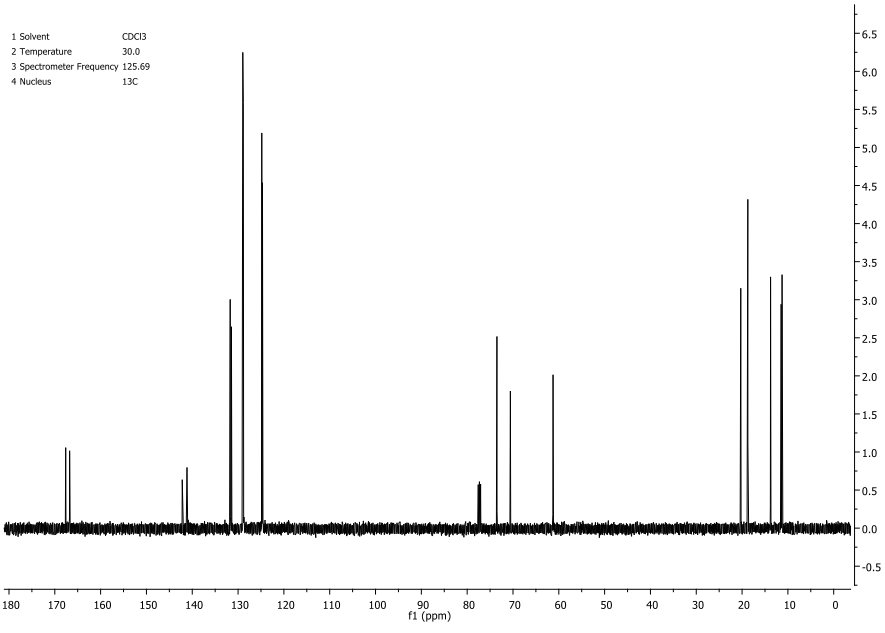
**1.7c**

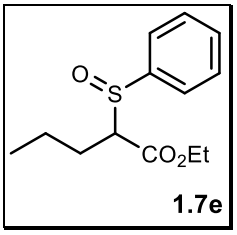

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
2.29
2.26
2.00
0.42
0.55
1.72
1.12
2.88
f1 (ppm)

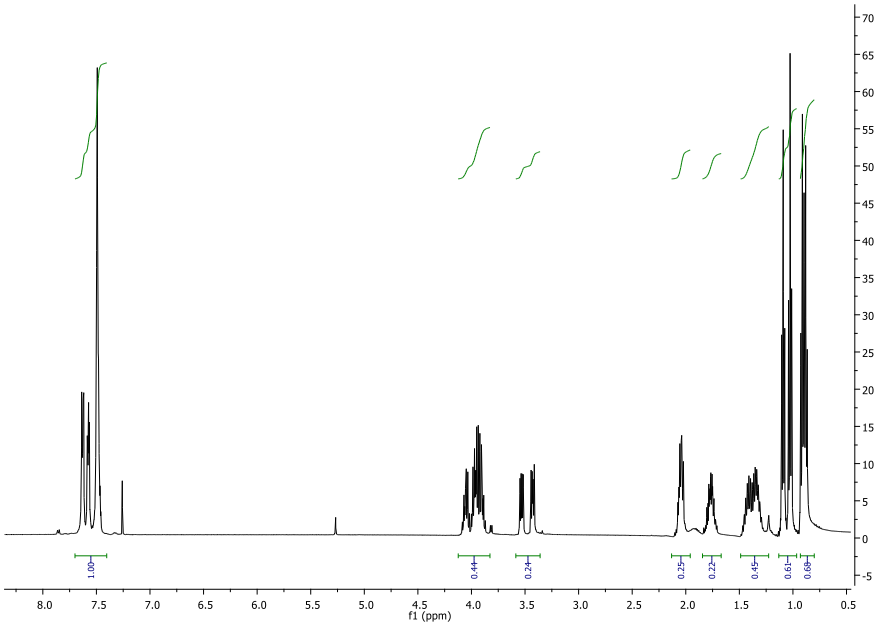

1 Solvent CDCl3
2 Temperature 30.0
3 Spectrometer Frequency 75.47
4 Nucleus 13C
f1 (ppm)

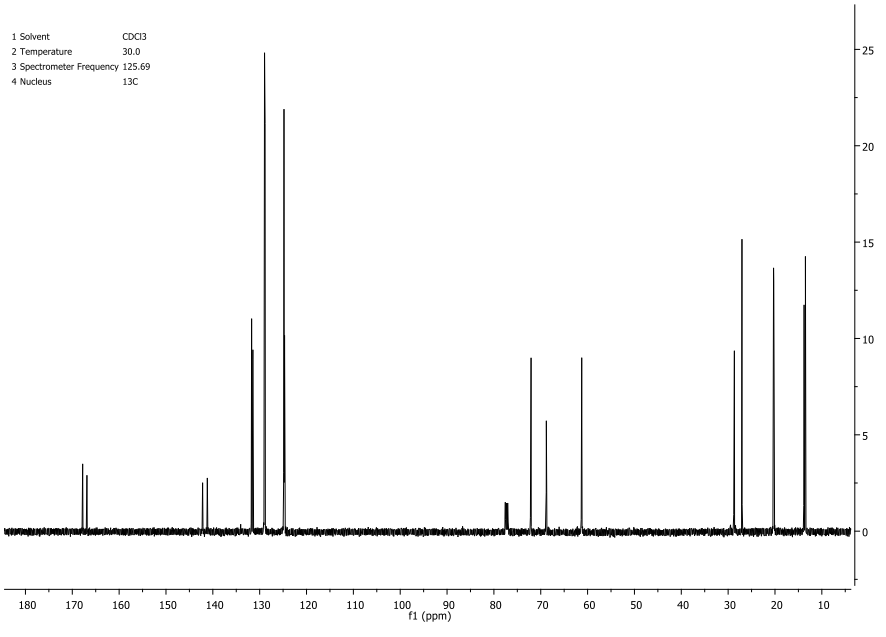

O
S
CO2Et
1.7d

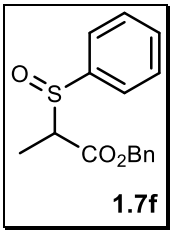

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.83
4 Nucleus 1H
f1 (ppm)


1 Solvent CDCl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

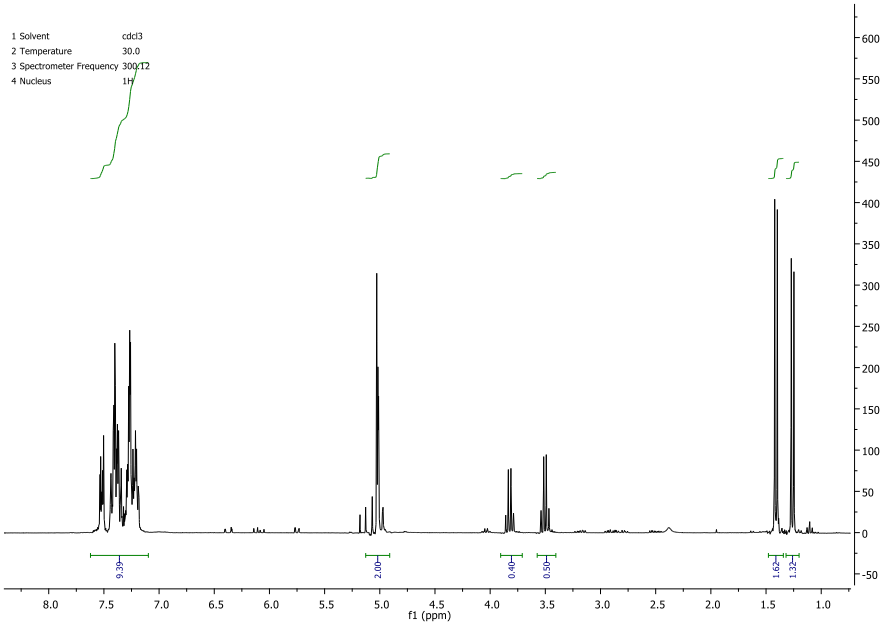

O
S
CO2Et
1.7e

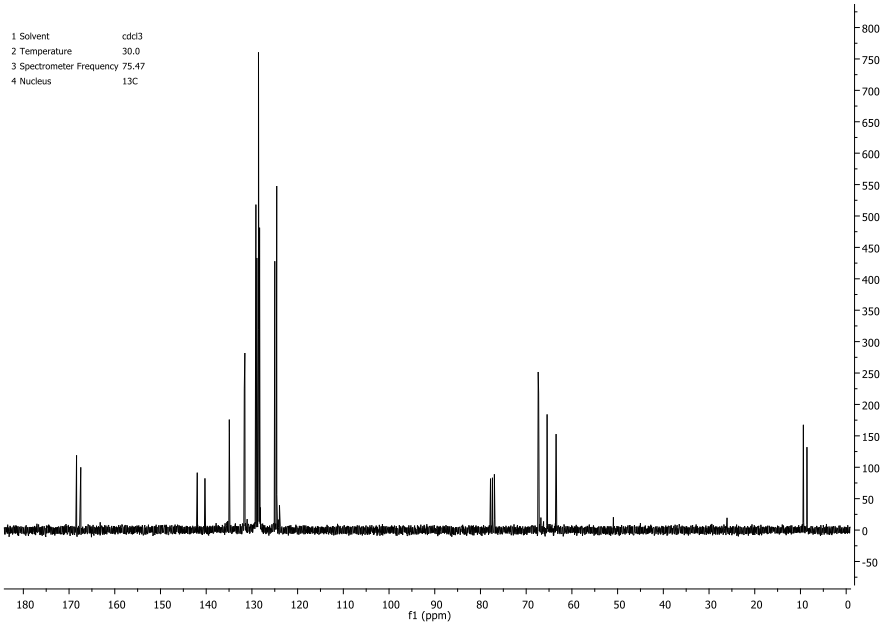

1.00
0.44
0.24
0.25
0.22
0.45
0.61
0.68
f1 (ppm)

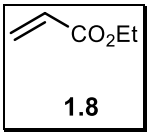

1 Solvent CDCl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)


O
S
CO2Bn

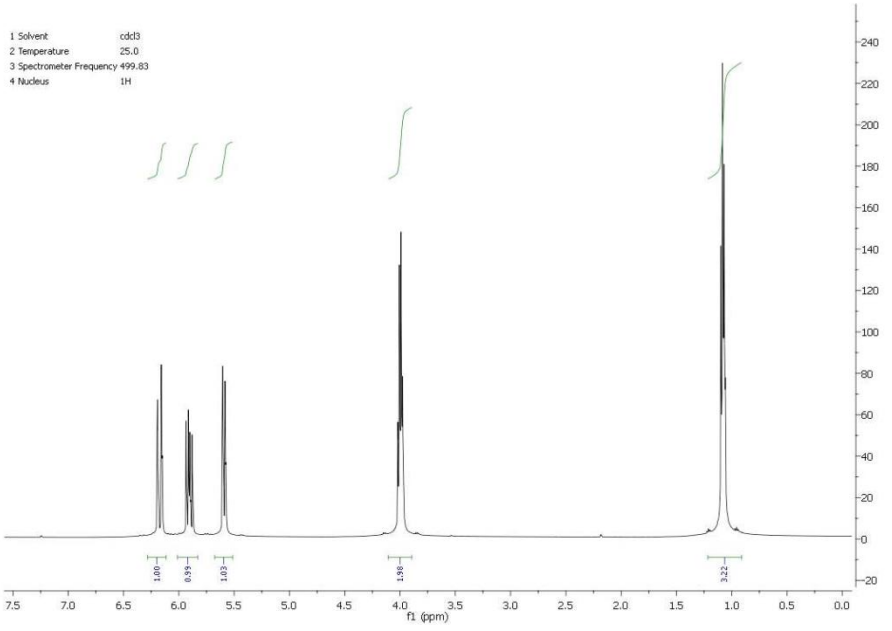
**1.7f**

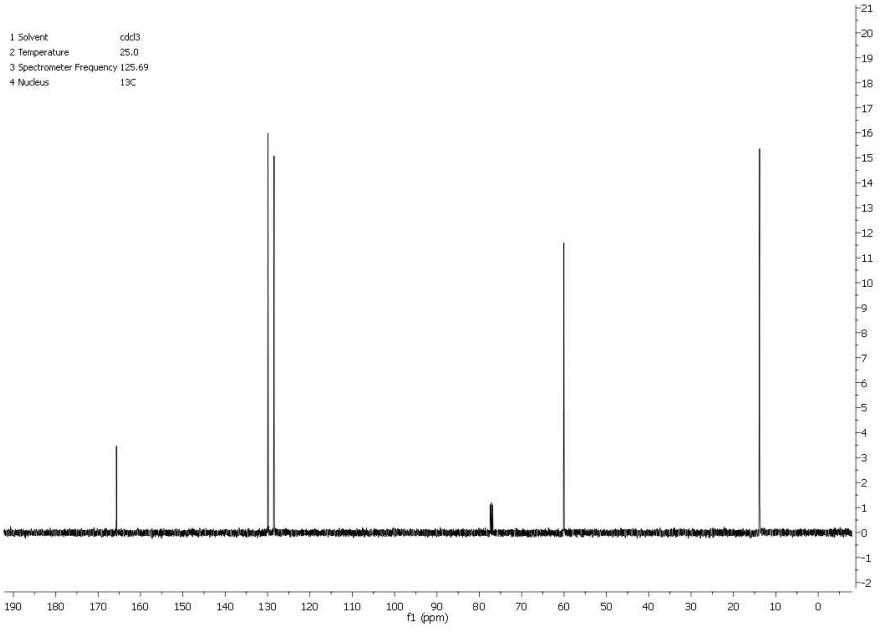

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 300.12
4 Nucleus 1H
9.39
2.00
0.40
0.50
1.62
1.32
f1 (ppm)

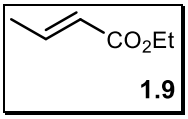

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 75.47
4 Nucleus 13C
f1 (ppm)


CO2Et

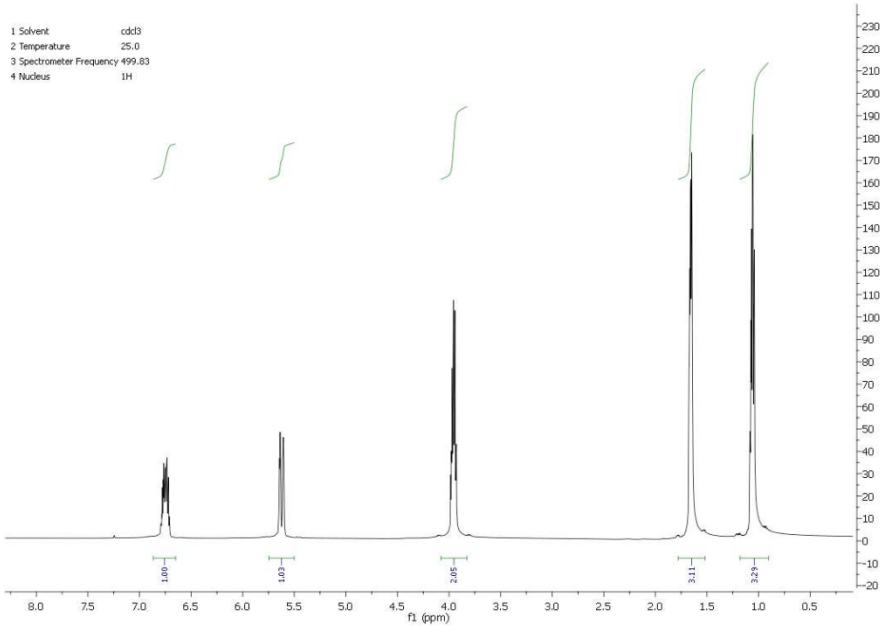
**1.8**

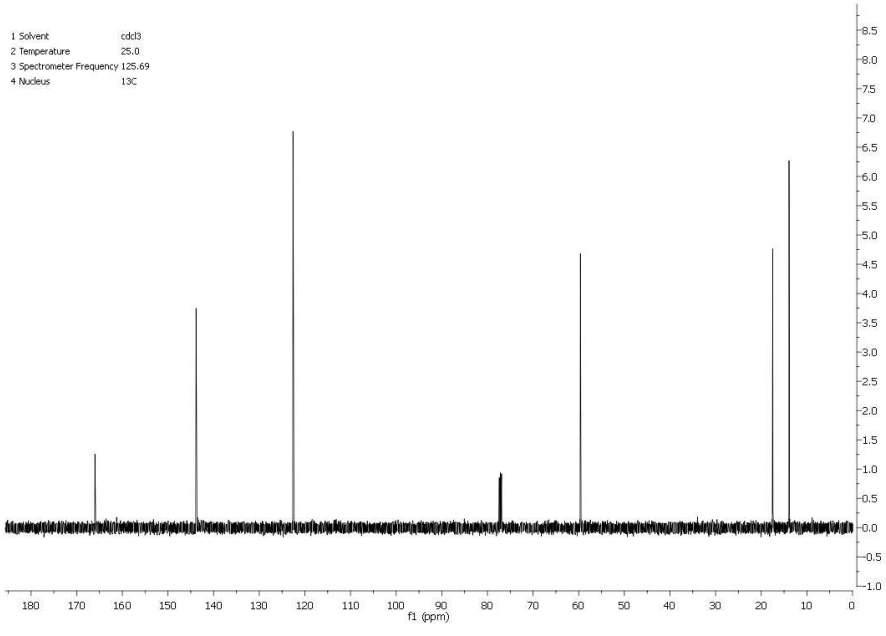

1 Solvent cdcl3
2 Temperature 25.0
3 Spectrometer Frequency 499.83
4 Nucleus 1H
1.00
0.99
1.03
1.98
3.22
f1 (ppm)

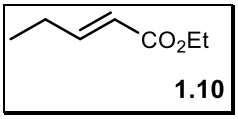

1 Solvent cdcl3
2 Temperature 25.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)


CO2Et

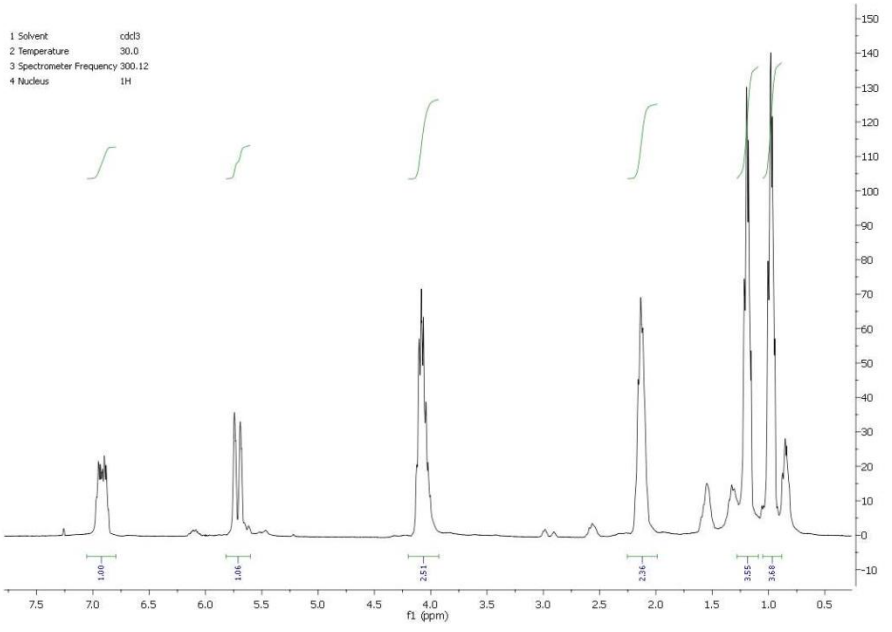
**1.9**

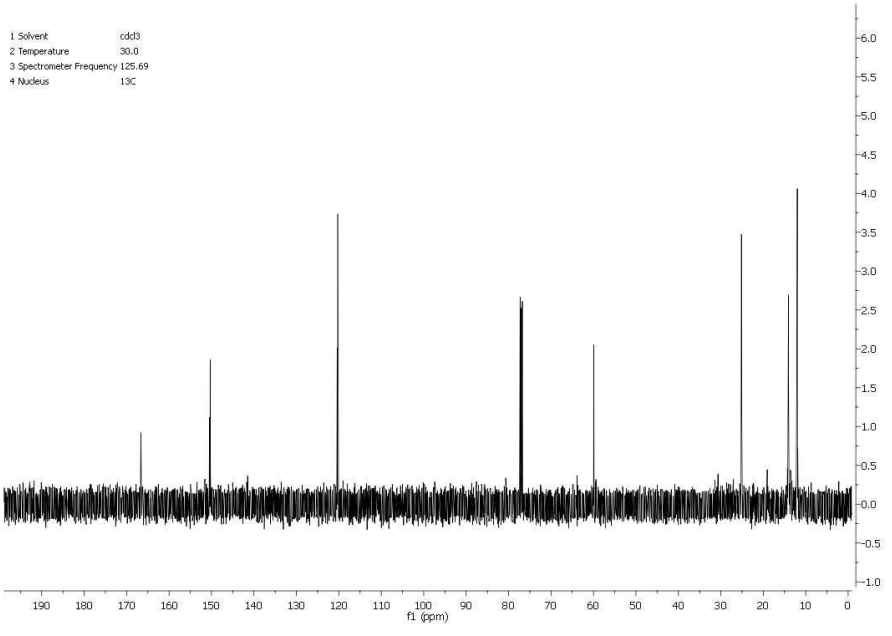

1 Solvent cdcl3
2 Temperature 25.0
3 Spectrometer Frequency 499.83
4 Nucleus 1H
1.00
1.03
2.05
3.11
3.23
f1 (ppm)

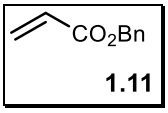

1 Solvent cdcl3
2 Temperature 25.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)


$CO_2Et$

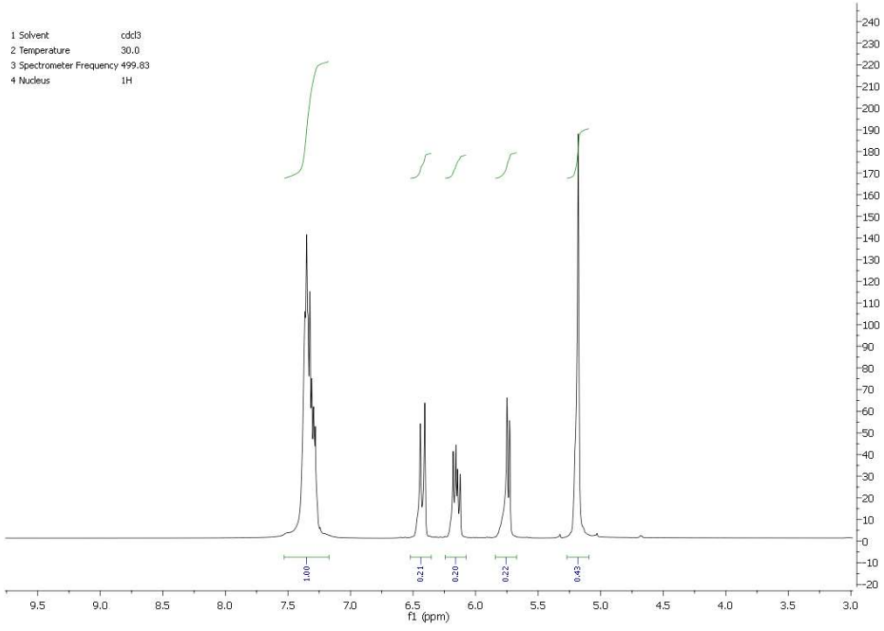
**1.10**

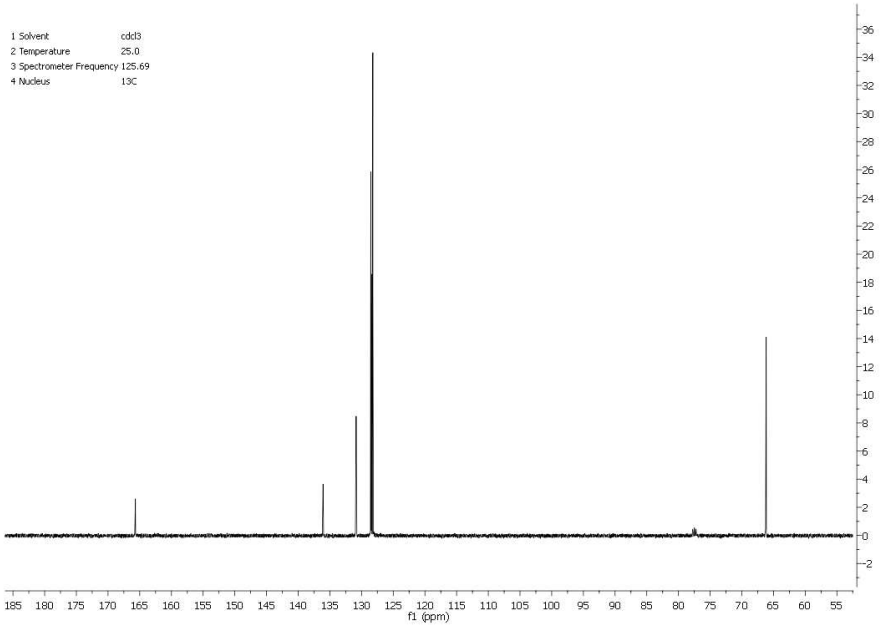

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 300.12
4 Nucleus 1H
1.00
1.06
2.51
2.36
3.55
3.68
f1 (ppm)

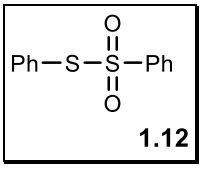

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

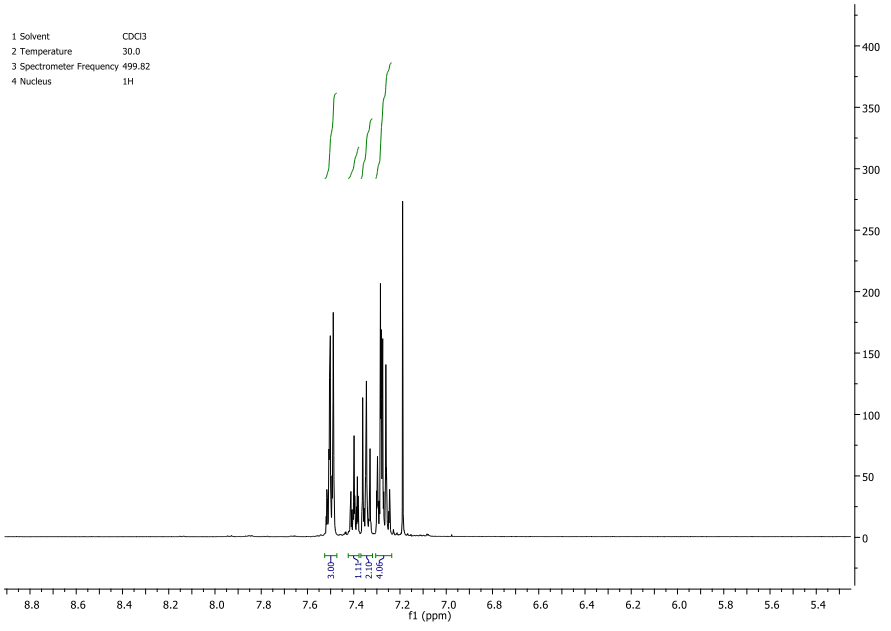

$CO_2Bn$

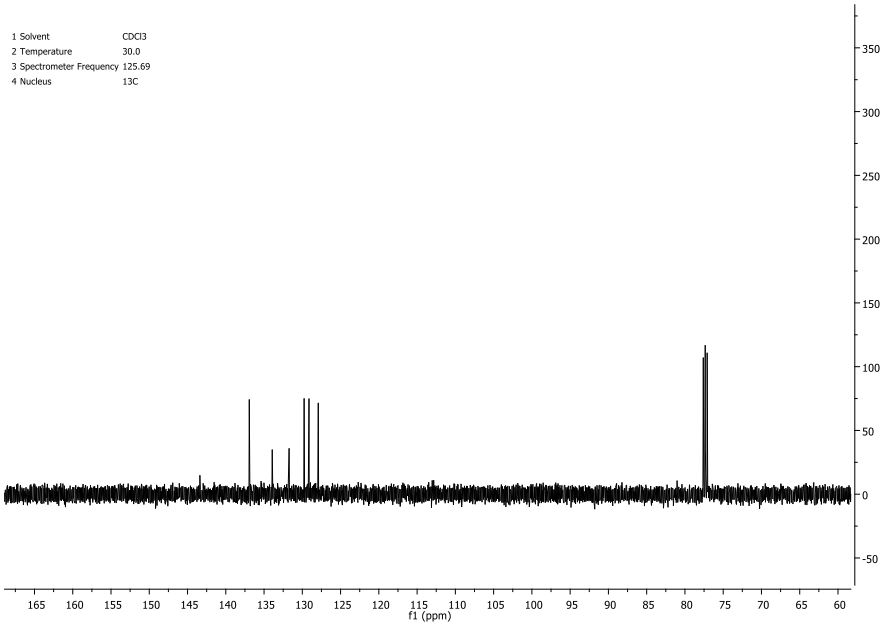
**1.11**

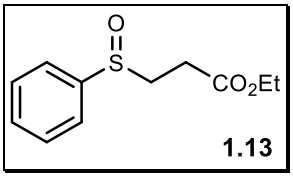

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.83
4 Nucleus 1H
1.00
0.21
0.20
0.22
0.43
f1 (ppm)


1 Solvent cdcl3
2 Temperature 25.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

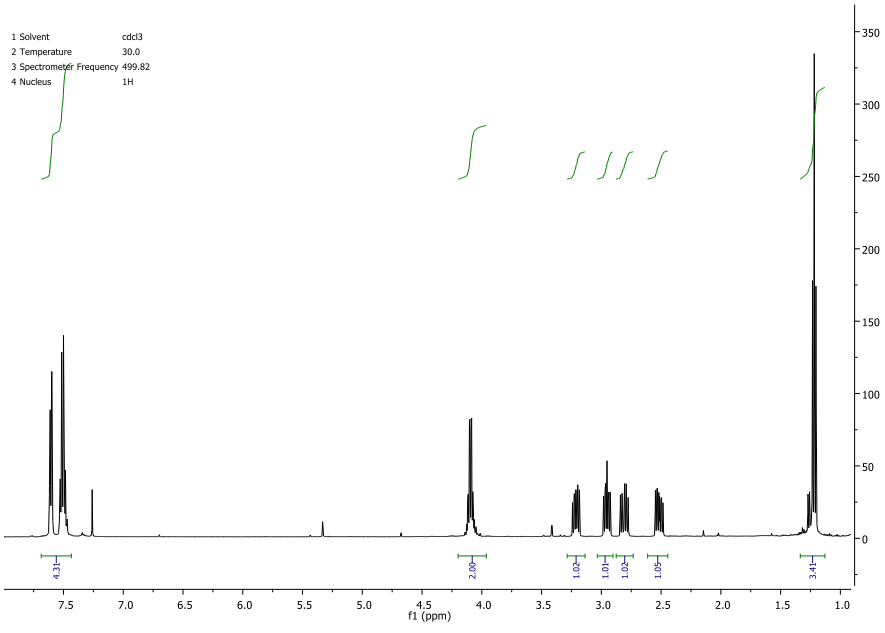

Ph—S—S—Ph
O
O
1.12

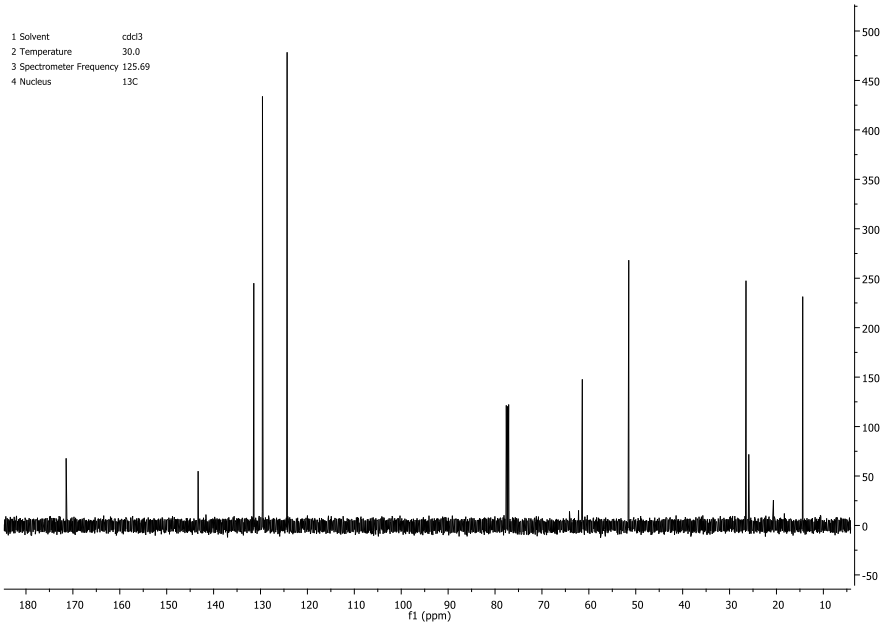

1 Solvent CDCl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
3.00
1.17
2.16
4.06
f1 (ppm)

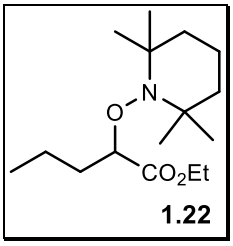

1 Solvent CDCl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)


O
S
CO2Et

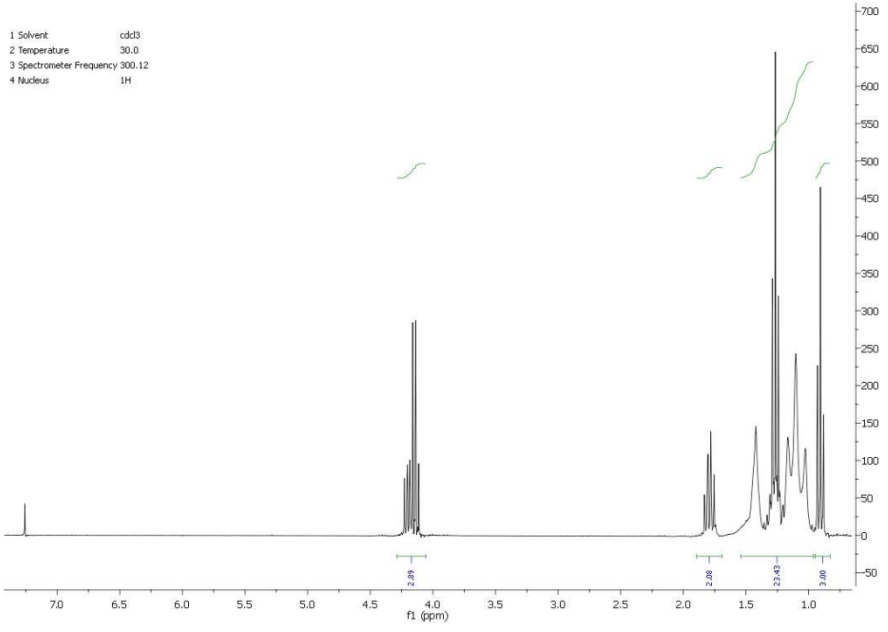
**1.13**

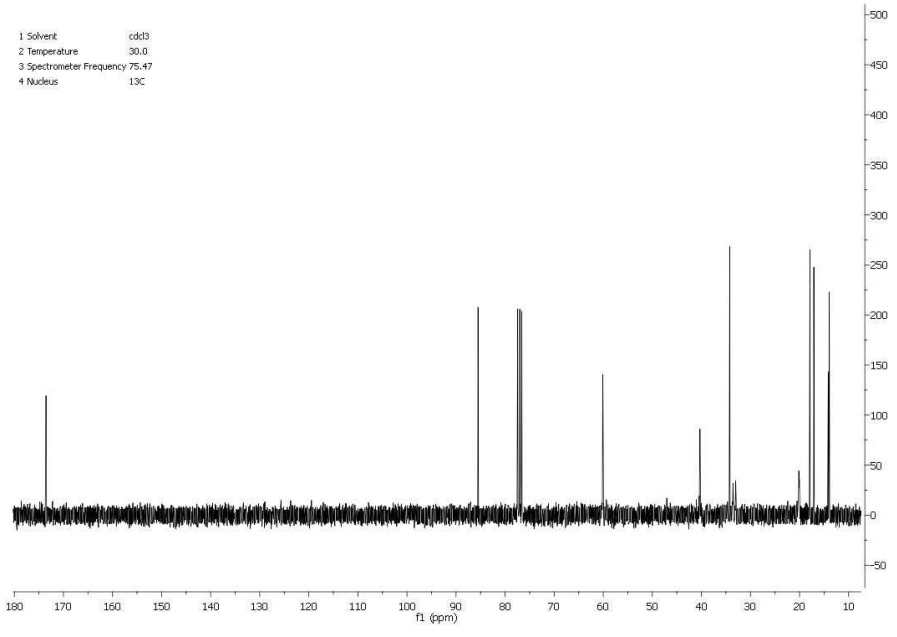

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
4.31
2.00
1.02
1.01
1.02
1.05
3.41
f1 (ppm)

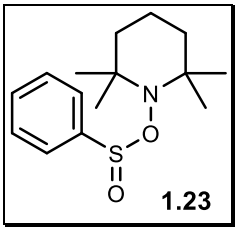

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

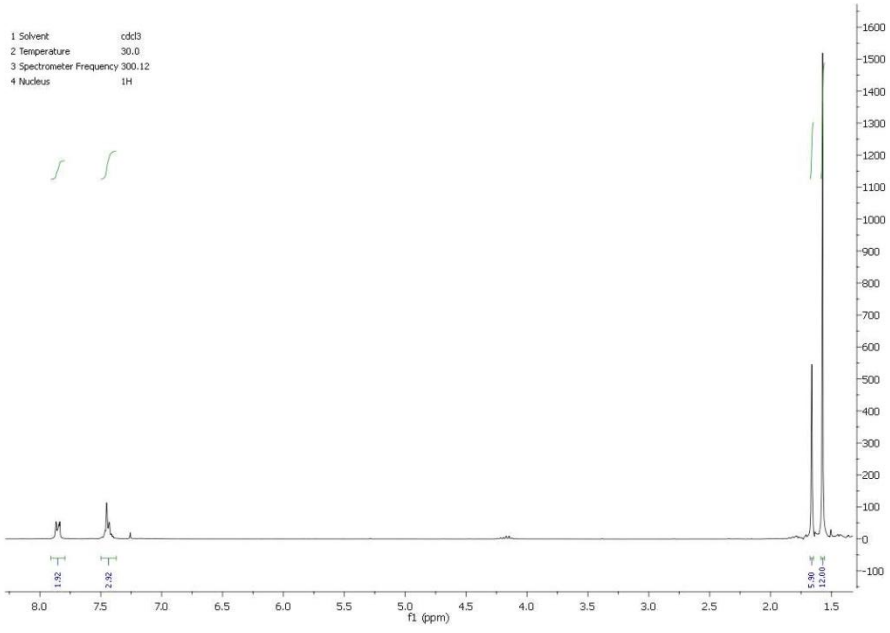

N
O
CO2Et

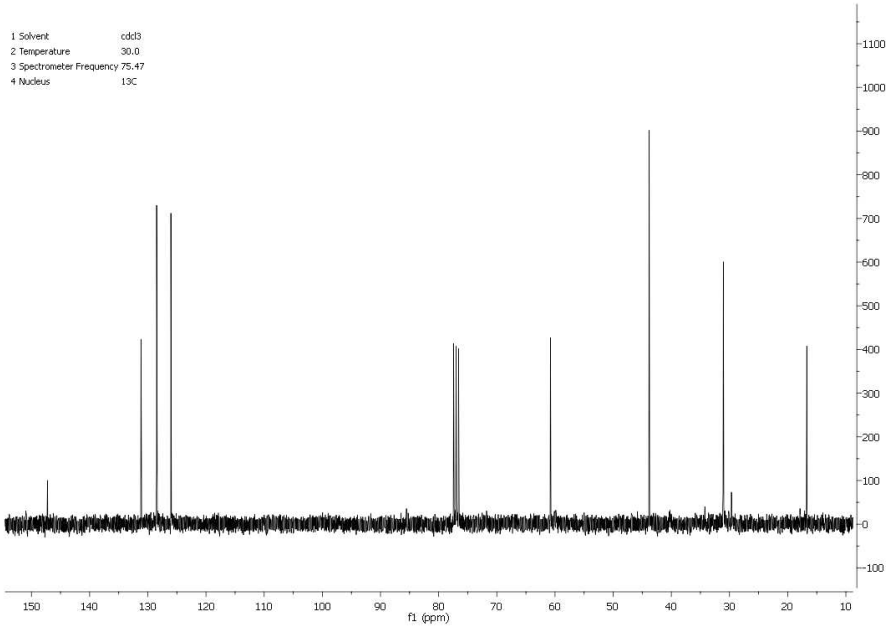
**1.22**

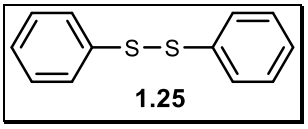

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 300.12
4 Nucleus 1H
2.89
2.00
23.43
3.00
f1 (ppm)


1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 75.47
4 Nucleus 13C
f1 (ppm)

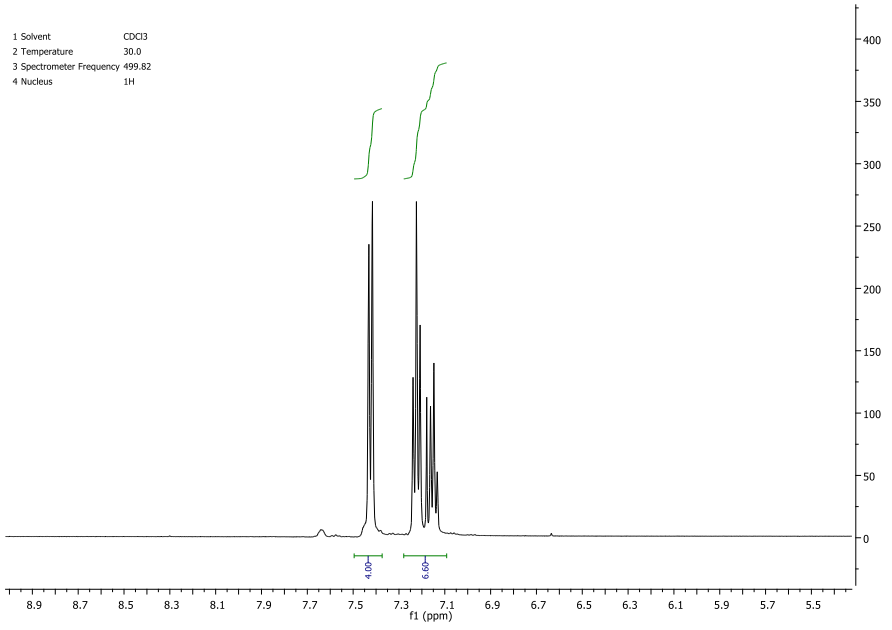

1.23

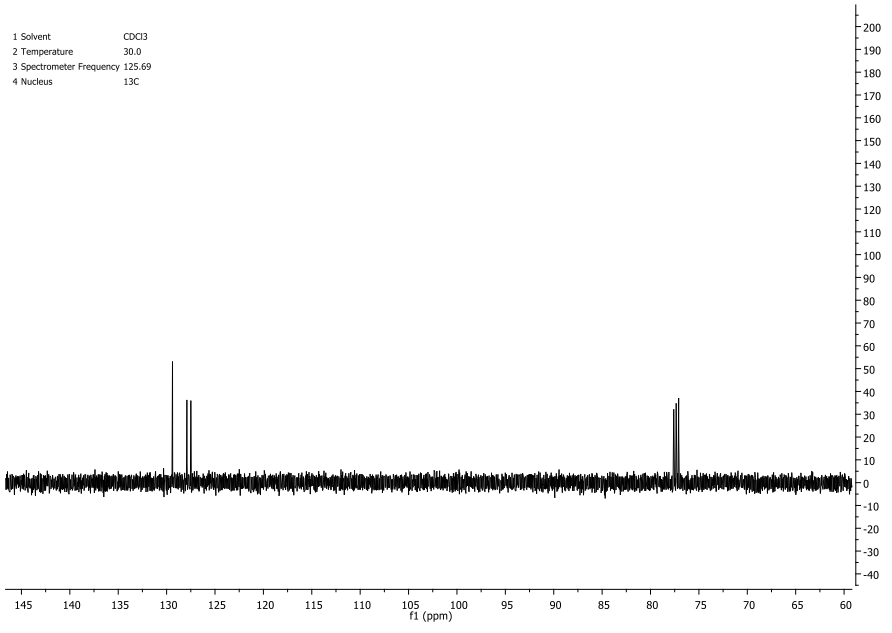

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 300.12
4 Nucleus 1H
1.92
2.92
5.90
12.00
f1 (ppm)


1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 75.47
4 Nucleus 13C
f1 (ppm)

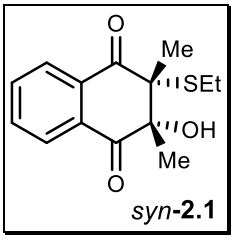

S–S

**1.25**

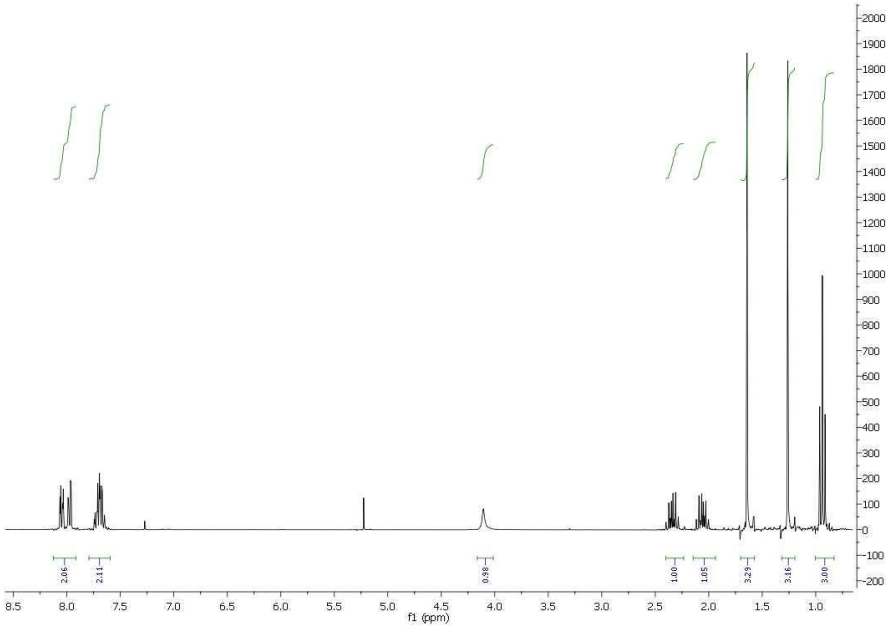

1 Solvent CDCl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
4.00
6.60
f1 (ppm)

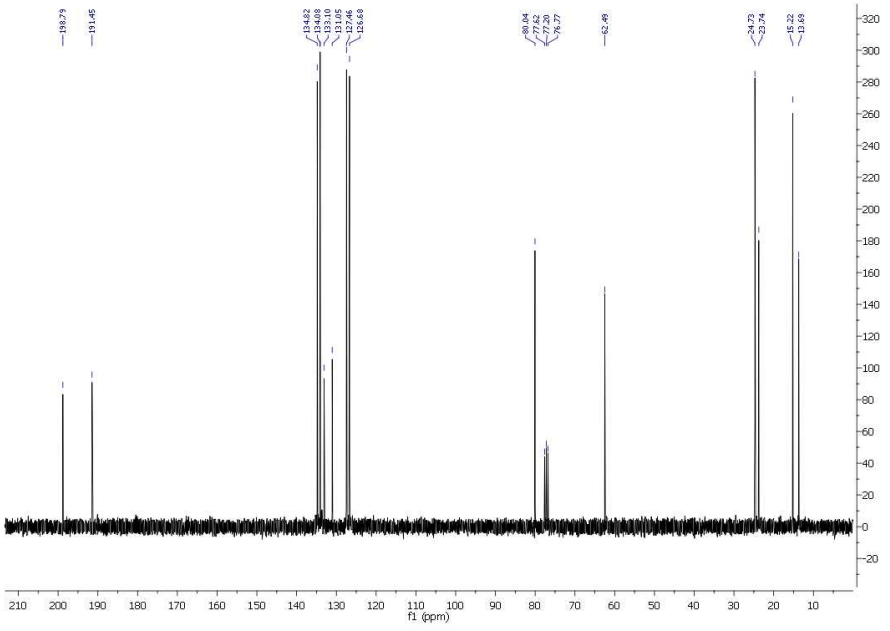

1 Solvent CDCl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

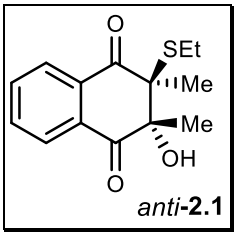
# Spectra chapter 2:


O
Me
SEt
OH
Me
O

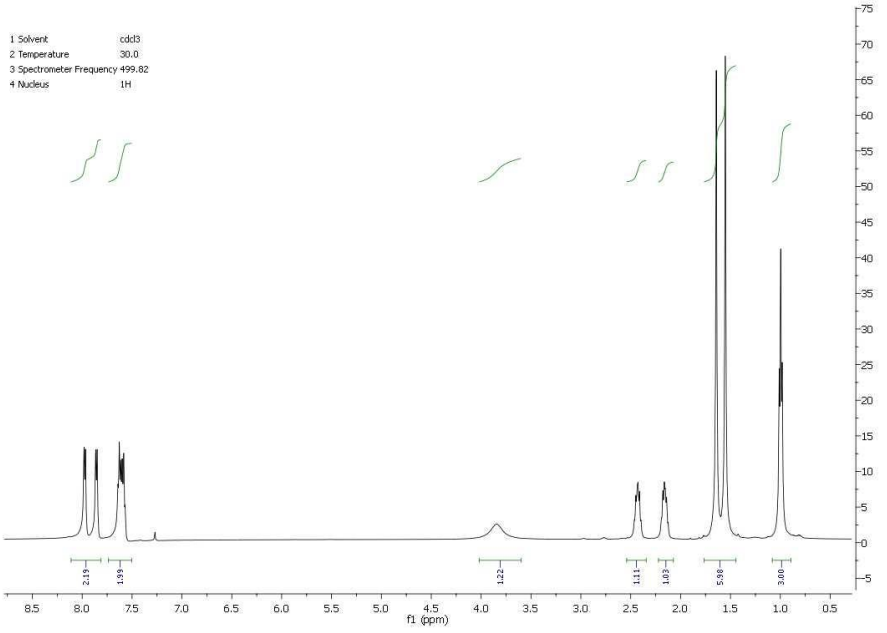
*syn*-**2.1**

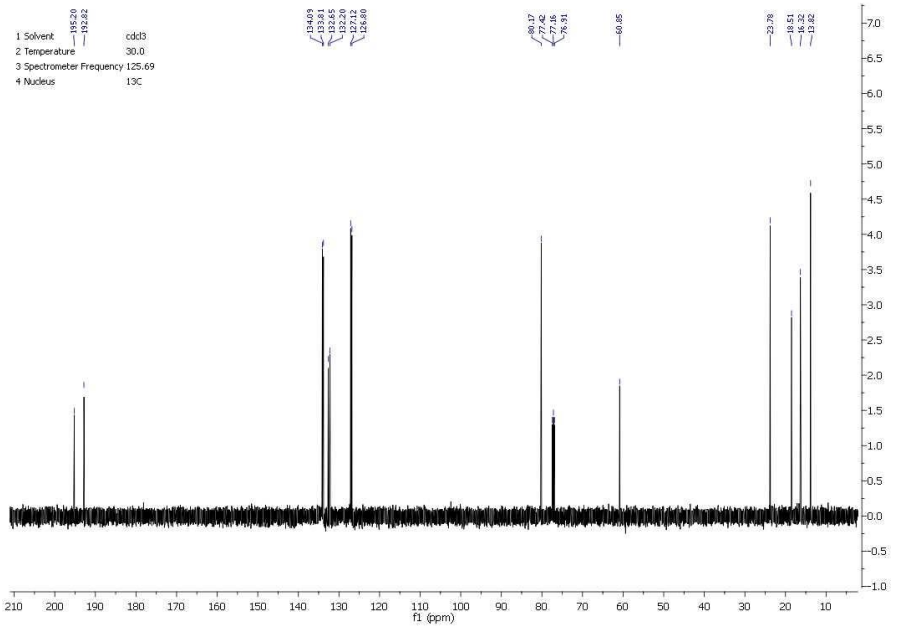

2.06
2.11
0.98
1.00
1.05
3.23
3.16
3.00
f1 (ppm)

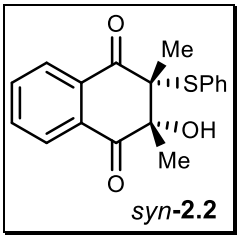

198.79
191.45
134.82
134.08
133.10
131.05
129.46
126.68
80.04
77.62
77.20
74.77
62.49
34.73
29.74
15.22
13.69
f1 (ppm)


O
SEt
Me
Me
OH
O

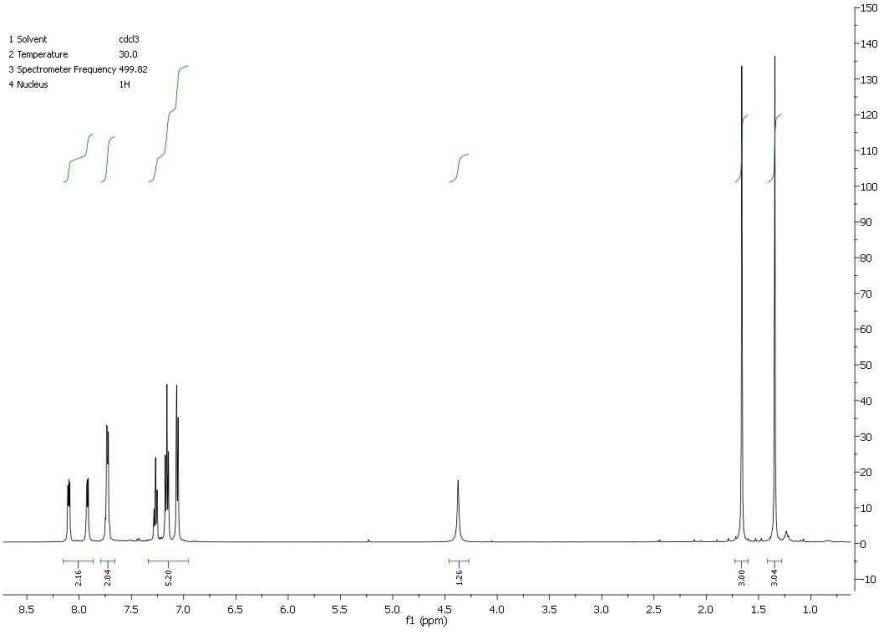
*anti*-**2.1**

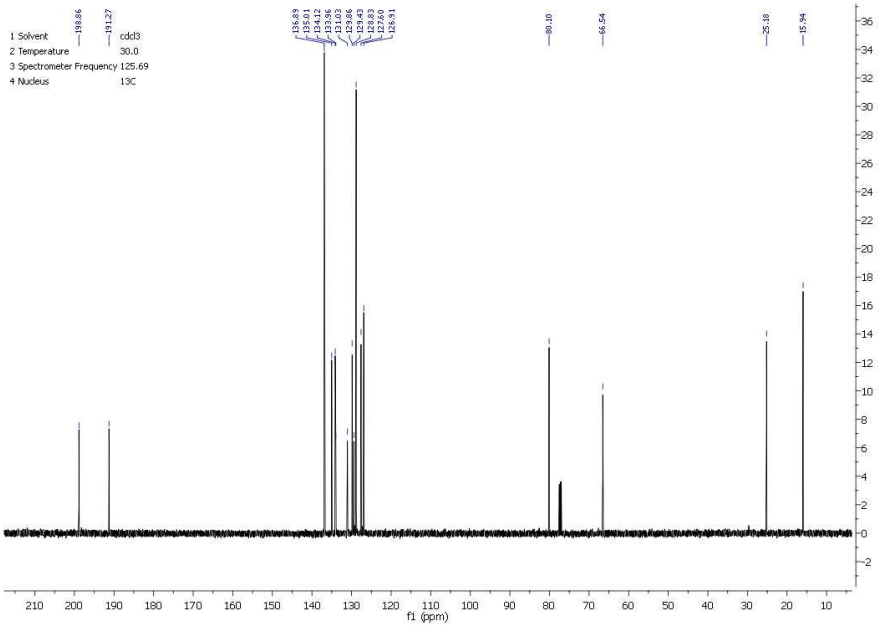

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

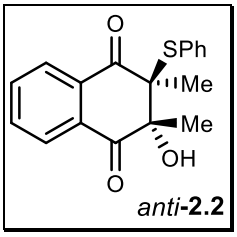

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)


O
Me
SPh
OH
Me
O

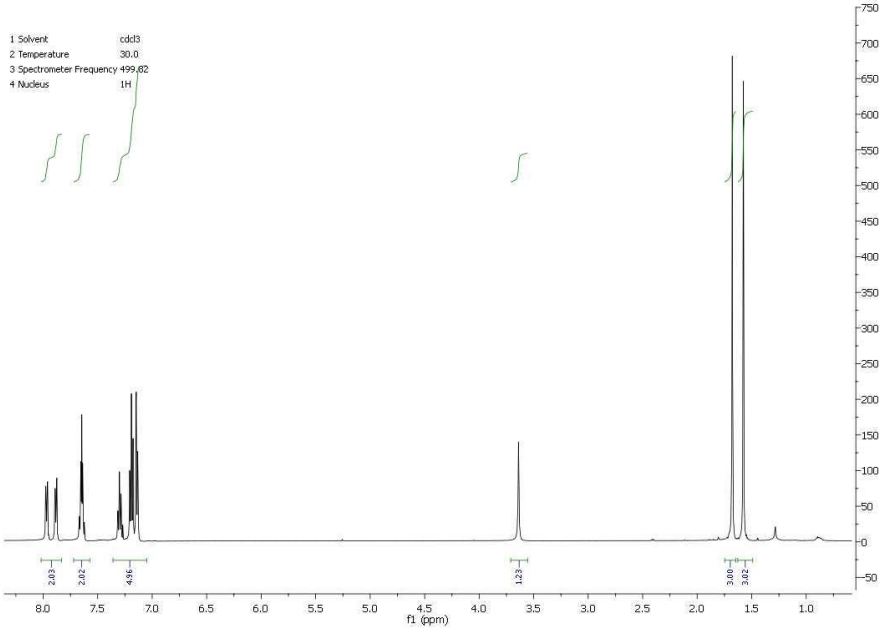
*syn*-**2.2**

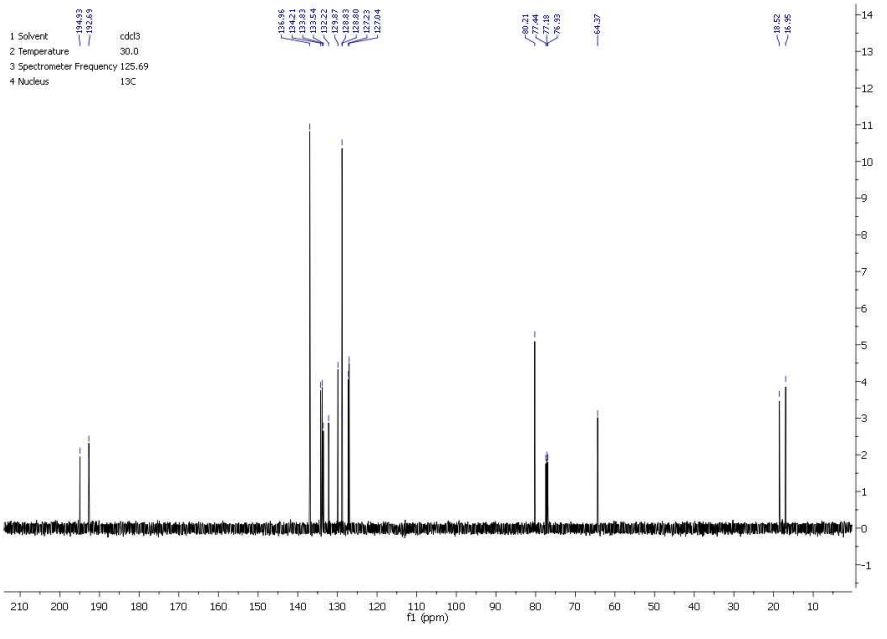

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
2.16
2.64
5.20
1.26
3.00
3.04
f1 (ppm)

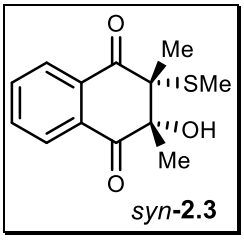

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
198.86
191.27
136.89
135.01
134.12
133.96
131.03
130.56
129.48
128.83
127.60
126.51
80.10
66.54
25.18
15.94
f1 (ppm)


O
SPh
Me
Me
OH
O

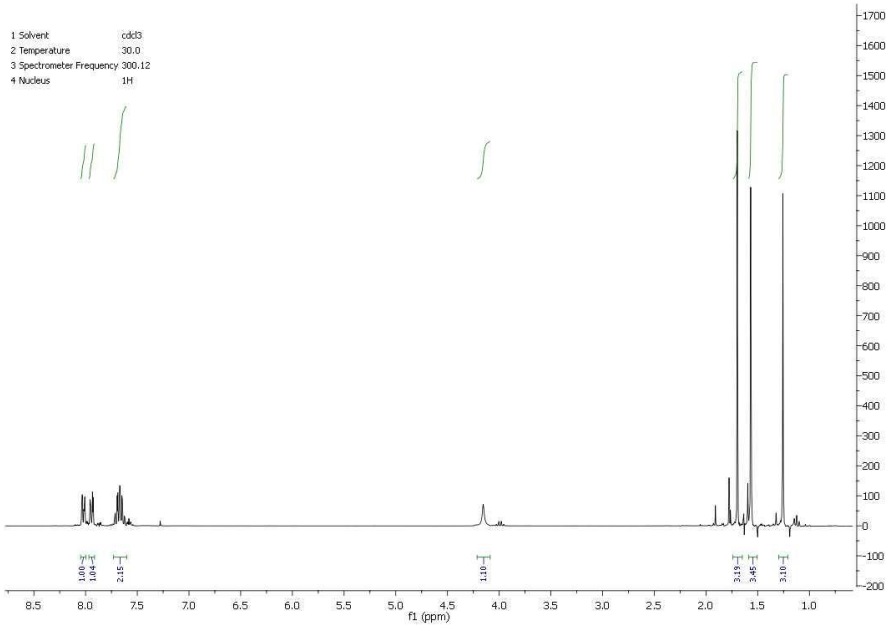
*anti*-**2.2**

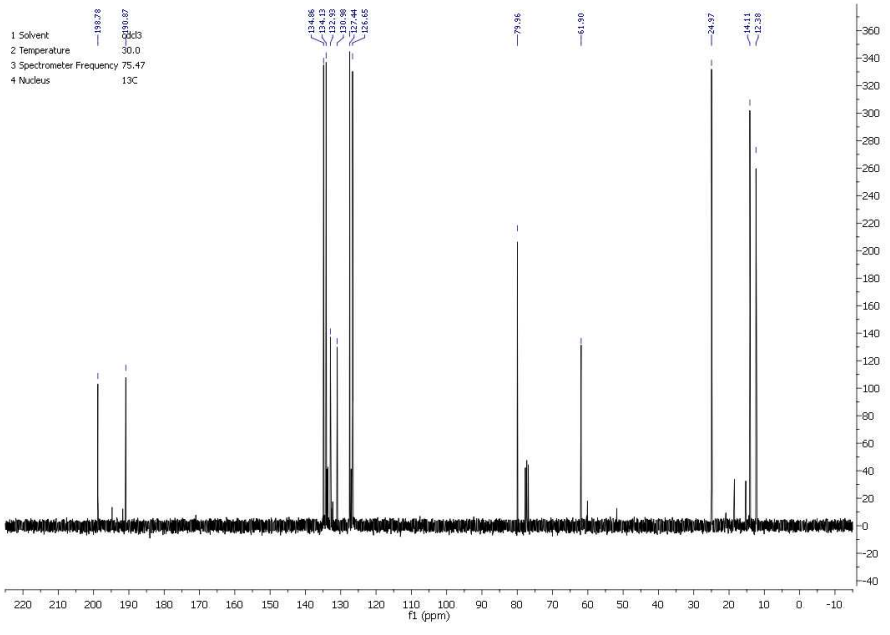

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
2.03
2.02
4.96
1.23
3.00
3.02
f1 (ppm)

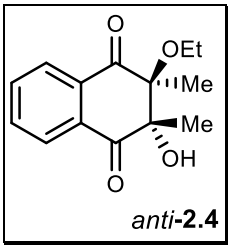

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
194.93
192.69
136.96
134.21
133.89
133.54
132.22
129.87
129.03
128.80
127.23
127.04
80.21
77.44
77.18
76.93
64.37
18.52
16.95
f1 (ppm)


O
Me
SMe
OH
Me
O

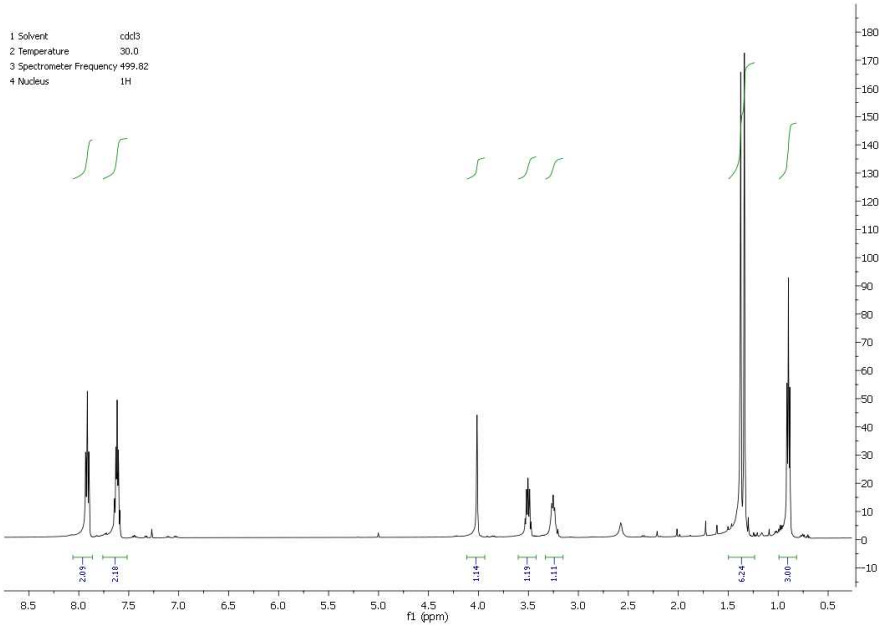
*syn*-**2.3**

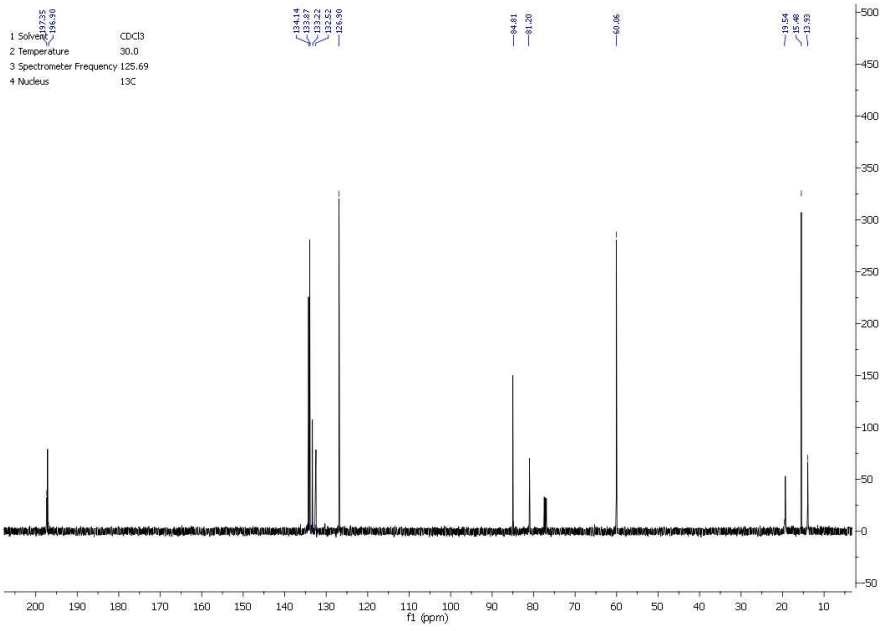

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 300.12
4 Nucleus 1H
f1 (ppm)

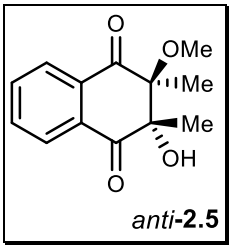

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 75.47
4 Nucleus 13C
f1 (ppm)


O
OEt
Me
Me
OH
O

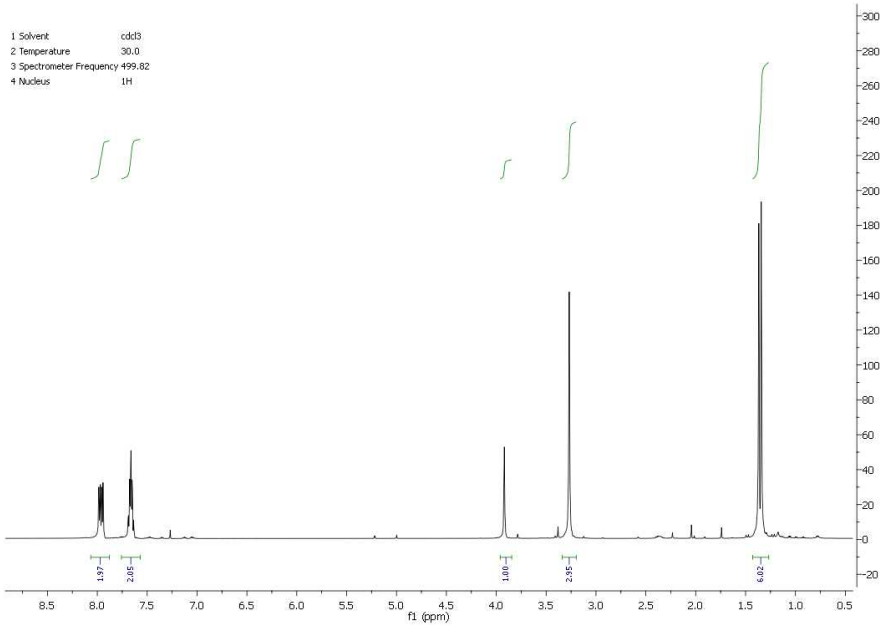
*anti*-**2.4**

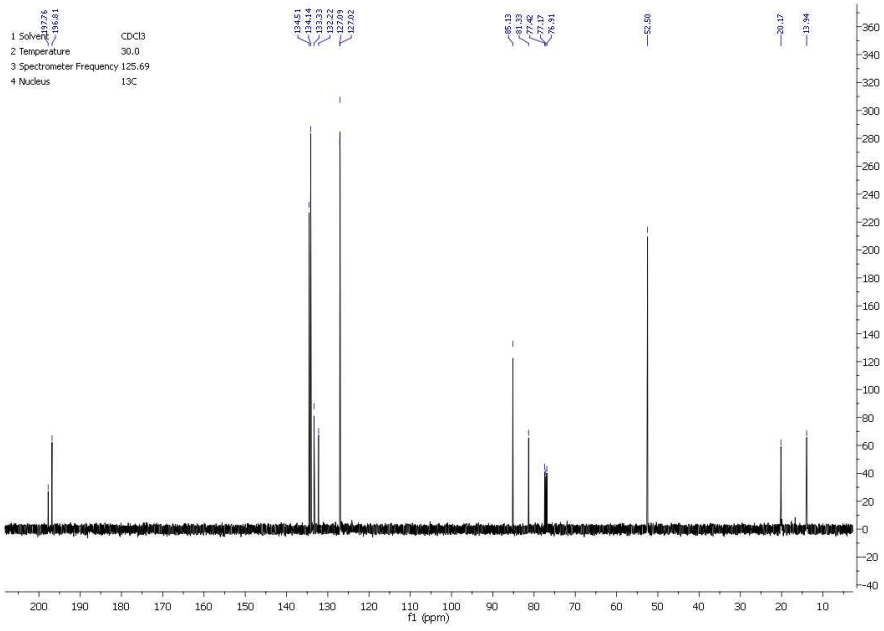

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)


1 Solvent CDCl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)


O
OMe
Me
Me
OH
O

*anti*-**2.5**


1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
1.97
2.05
1.00
2.95
6.02
f1 (ppm)


1 Solvent CDCl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
197.76
196.81
134.51
134.14
133.33
132.22
127.09
127.02
86.13
81.33
77.46
77.17
76.91
52.50
20.17
13.94
f1 (ppm)

## Crystallographic data

**Table 1** Crystal data and structure refinement for *syn*-**2.2**

| | |
|---|---|
| Identification code | STR1168M |
| Empirical formula | $C_{18}H_{16}O_3S$ |
| Formula weight | 312.37 |
| Temperature/K | 298(2) |
| Crystal system | triclinic |
| Space group | P-1 |
| a/Å | 7.7667(5) |
| b/Å | 10.1556(6) |
| c/Å | 10.4484(6) |
| α/° | 77.2900(10) |
| β/° | 71.4970(10) |
| γ/° | 80.5300(10) |
| Volume/Å$^3$ | 758.37(8) |
| Z | 2 |
| $\rho_{calc}$mg/mm$^3$ | 1.368 |
| m/mm$^{-1}$ | 0.223 |
| F(000) | 328.0 |

| | |
|---|---|
| Crystal size/mm$^3$ | 0.24 × 0.22 × 0.16 |
| 2Θ range for data collection | 4.14 to 54.98° |
| Index ranges | -10 ≤ h ≤ 10, -13 ≤ k ≤ 13, -13 ≤ l ≤ 13 |
| Reflections collected | 8857 |
| Independent reflections | 3482[R(int) = 0.0164] |
| Data/restraints/parameters | 3482/0/263 |
| Goodness-of-fit on $F^2$ | 1.027 |
| Final R indexes [I>=2σ (I)] | $R_1$ = 0.0374, $wR_2$ = 0.1030 |
| Final R indexes [all data] | $R_1$ = 0.0458, $wR_2$ = 0.1110 |
| Largest diff. peak/hole / e Å$^{-3}$ | 0.27/-0.18 |

**Table 2** Fractional Atomic Coordinates (×10$^4$) and Equivalent Isotropic Displacement Parameters (Å$^2$×10$^3$) for STR1168M. $U_{eq}$ is defined as 1/3 of of the trace of the orthogonalised $U_{IJ}$ tensor.

| Atom | *x* | *y* | *z* | U(eq) |
|---|---|---|---|---|
| S1 | 654.0(6) | 2628.1(4) | 9242.9(4) | 55.90(15) |
| C1 | 834.5(19) | 3369.1(14) | 7417.0(15) | 45.5(3) |
| O1 | -1738.3(15) | 3088.2(13) | 6771.5(14) | 64.0(3) |
| O2 | 4923.6(15) | 1140.8(13) | 6980.0(17) | 76.4(4) |

| | | | | |
|---|---|---|---|---|
| C2 | 2877.7(19) | 3090.4(15) | 6623.8(17) | 48.8(3) |
| C3 | 171(3) | 4869.6(17) | 7261(2) | 61.6(4) |
| O3 | 3927.2(18) | 3727.0(14) | 7141.4(16) | 68.8(4) |
| C4 | 3230(3) | 3618(2) | 5083(2) | 64.0(5) |
| C5 | -303.7(17) | 2578.0(14) | 6974.6(14) | 43.4(3) |
| C6 | 3454.2(18) | 1568.8(15) | 6800.6(17) | 49.2(3) |
| C7 | 2215.8(17) | 674.4(14) | 6693.5(15) | 42.9(3) |
| C8 | 2855(2) | -644.6(16) | 6497.7(18) | 54.1(4) |
| C9 | 1687(3) | -1497.6(18) | 6451.5(19) | 59.7(4) |
| C10 | -129(3) | -1042.6(18) | 6608.5(17) | 58.8(4) |
| C11 | -783(2) | 271.0(17) | 6780.7(16) | 51.1(4) |
| C12 | 382.9(17) | 1151.3(14) | 6814.4(13) | 41.0(3) |
| C13 | -1746(2) | 2683.1(16) | 10000.3(15) | 51.7(4) |
| C14 | -2687(3) | 3776.9(18) | 10611.0(17) | 58.1(4) |
| C15 | -4542(3) | 3826(2) | 11224.3(19) | 70.4(5) |
| C16 | -5478(3) | 2811(2) | 11214(2) | 78.8(6) |
| C17 | -4573(3) | 1719(3) | 10633(2) | 82.3(6) |
| C18 | -2700(3) | 1630(2) | 10039.7(19) | 68.5(5) |

**Table 3** Anisotropic Displacement Parameters ($Å^2 \times 10^3$) for *syn*-**2.2**. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+...+2hka \times b \times U_{12}]$

| Atom | $U_{11}$ | $U_{22}$ | $U_{33}$ | $U_{23}$ | $U_{13}$ | $U_{12}$ |
|---|---|---|---|---|---|---|
| S1 | 58.7(3) | 58.0(2) | 54.4(2) | -6.35(17) | -26.36(18) | -0.97(18) |
| C1 | 42.3(7) | 41.2(7) | 53.3(8) | -1.8(6) | -19.0(6) | -3.9(5) |
| O1 | 42.9(6) | 66.8(7) | 86.3(8) | -6.1(6) | -34.0(6) | 3.8(5) |
| O2 | 38.6(6) | 62.1(7) | 133.8(12) | -6.4(7) | -40.3(7) | -4.4(5) |
| C2 | 37.5(7) | 43.6(7) | 66.7(9) | -2.7(6) | -18.6(6) | -11.3(6) |
| C3 | 67.0(11) | 41.9(8) | 75.5(12) | -4.3(8) | -27.6(10) | 0.7(7) |
| O3 | 54.0(7) | 56.7(7) | 108.3(11) | -13.2(7) | -36.0(7) | -18.1(6) |
| C4 | 52.9(9) | 59(1) | 68.0(11) | 6.3(8) | -8.7(8) | -14.1(8) |
| C5 | 33.3(6) | 49.7(7) | 45.0(7) | -0.2(6) | -13.6(5) | -4.7(5) |
| C6 | 31.4(6) | 48.4(8) | 66.7(9) | -2.7(7) | -16.6(6) | -7.2(5) |
| C7 | 34.9(6) | 43.6(7) | 49.0(7) | -2.1(6) | -13.3(5) | -6.8(5) |
| C8 | 48.0(8) | 47.4(8) | 63.8(9) | -6.3(7) | -15.2(7) | -3.4(6) |
| C9 | 70.7(11) | 45.9(8) | 62.2(10) | -8.5(7) | -16.3(8) | -13.4(8) |
| C10 | 65.6(10) | 61.9(10) | 54.1(9) | -7.7(7) | -14.9(7) | -29.8(8) |
| C11 | 40.3(7) | 66.7(10) | 49.0(8) | -5.5(7) | -14.6(6) | -16.7(7) |
| C12 | 33.7(6) | 49.0(7) | 40.7(7) | -2.3(5) | -12.7(5) | -9.1(5) |
| C13 | 64.1(9) | 49.5(8) | 41.5(7) | -4.9(6) | -15.7(7) | -10.0(7) |

| | | | | | | |
|---|---|---|---|---|---|---|
| C14 | 73.4(11) | 51.5(9) | 50.5(9) | -8.6(7) | -19.0(8) | -8.6(8) |
| C15 | 77.2(12) | 65.0(11) | 56.5(10) | -8.8(8) | -7.4(9) | -0.2(9) |
| C16 | 71.8(12) | 86.9(14) | 64.6(12) | -7.1(10) | -1.7(10) | -16.7(11) |
| C17 | 90.4(15) | 85.7(15) | 66.7(12) | -11.3(10) | -0.8(10) | -45.1(12) |
| C18 | 87.0(13) | 57.2(10) | 55.1(10) | -14.5(8) | -1.1(9) | -23.8(9) |

**Table 4** Bond Lengths for *syn*-**2.2**

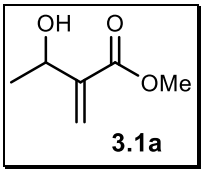
| Atom | Atom | Length/Å | Atom | Atom | Length/Å |
|---|---|---|---|---|---|
| S1 | C1 | 1.8597(15) | C7 | C8 | 1.386(2) |
| S1 | C13 | 1.7753(17) | C7 | C12 | 1.4011(18) |
| C1 | C2 | 1.551(2) | C8 | C9 | 1.372(2) |
| C1 | C3 | 1.516(2) | C9 | C10 | 1.379(3) |
| C1 | C5 | 1.516(2) | C10 | C11 | 1.376(2) |
| O1 | C5 | 1.2110(16) | C11 | C12 | 1.386(2) |
| O2 | C6 | 1.2096(17) | C13 | C14 | 1.387(2) |
| C2 | O3 | 1.4078(18) | C13 | C18 | 1.385(2) |
| C2 | C4 | 1.531(2) | C14 | C15 | 1.376(3) |
| C2 | C6 | 1.524(2) | C15 | C16 | 1.359(3) |
| C5 | C12 | 1.486(2) | C16 | C17 | 1.364(3) |
| C6 | C7 | 1.4708(19) | C17 | C18 | 1.385(3) |

**Table 5** Bond Angles for *syn*-**2.2**

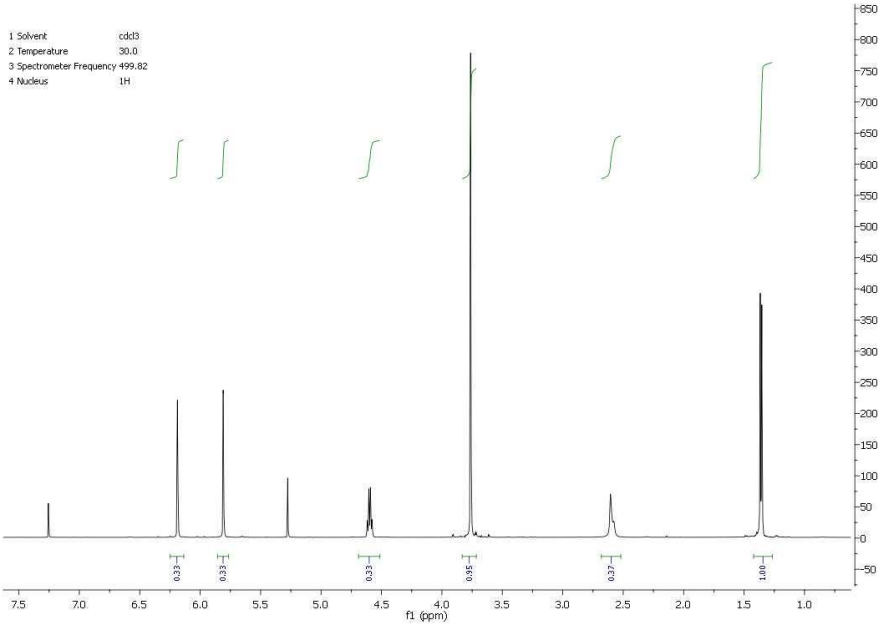
| Atom | Atom | Atom | Angle/° | Atom | Atom | Atom | Angle/° |
|---|---|---|---|---|---|---|---|
| C13 | S1 | C1 | 102.01(7) | C8 | C7 | C6 | 119.98(13) |
| C2 | C1 | S1 | 105.37(9) | C8 | C7 | C12 | 120.15(13) |
| C3 | C1 | S1 | 110.66(12) | C12 | C7 | C6 | 119.86(13) |
| C3 | C1 | C2 | 112.65(13) | C9 | C8 | C7 | 120.10(15) |
| C5 | C1 | S1 | 106.50(9) | C8 | C9 | C10 | 119.87(16) |
| C5 | C1 | C2 | 109.53(12) | C11 | C10 | C9 | 120.84(15) |
| C5 | C1 | C3 | 111.76(13) | C10 | C11 | C12 | 120.06(14) |
| O3 | C2 | C1 | 108.69(13) | C7 | C12 | C5 | 120.66(12) |
| O3 | C2 | C4 | 109.95(14) | C11 | C12 | C5 | 120.41(12) |
| O3 | C2 | C6 | 109.80(12) | C11 | C12 | C7 | 118.94(14) |
| C4 | C2 | C1 | 111.33(13) | C14 | C13 | S1 | 119.78(13) |
| C6 | C2 | C1 | 110.20(11) | C18 | C13 | S1 | 121.34(14) |
| C6 | C2 | C4 | 106.86(14) | C18 | C13 | C14 | 118.84(17) |
| O1 | C5 | C1 | 121.04(13) | C15 | C14 | C13 | 120.62(18) |
| O1 | C5 | C12 | 120.99(13) | C16 | C15 | C14 | 120.10(19) |
| C12 | C5 | C1 | 117.96(11) | C15 | C16 | C17 | 120.1(2) |
| O2 | C6 | C2 | 119.49(13) | C16 | C17 | C18 | 120.8(2) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| O2 | C6 | C7 | 122.28(14) | C13 | C18 | C17 | 119.41(19) |
| C7 | C6 | C2 | 118.19(11) | | | | |

# Spectra Chapter 3:


OH
O
OMe

**3.1a**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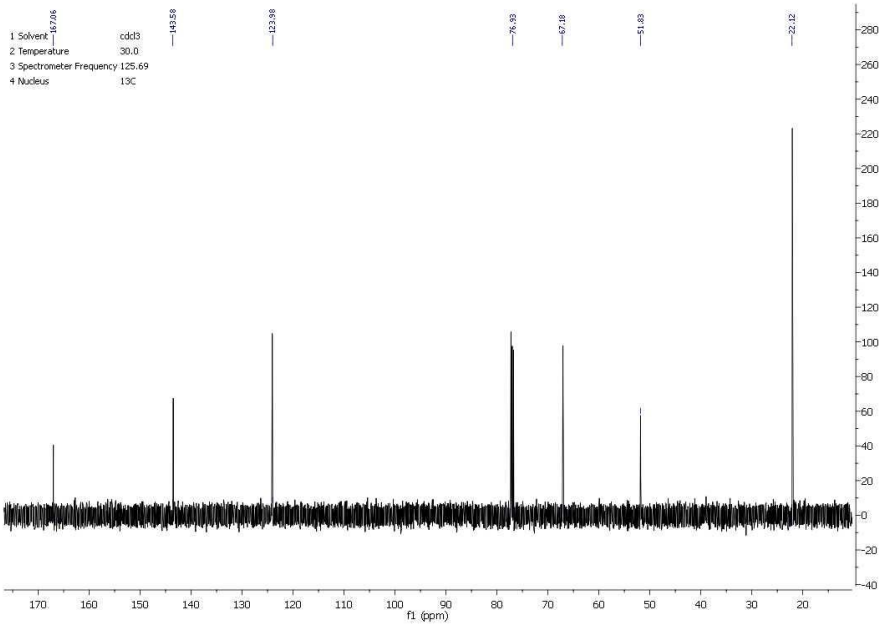
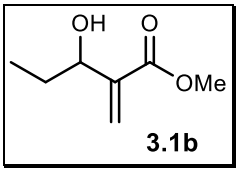
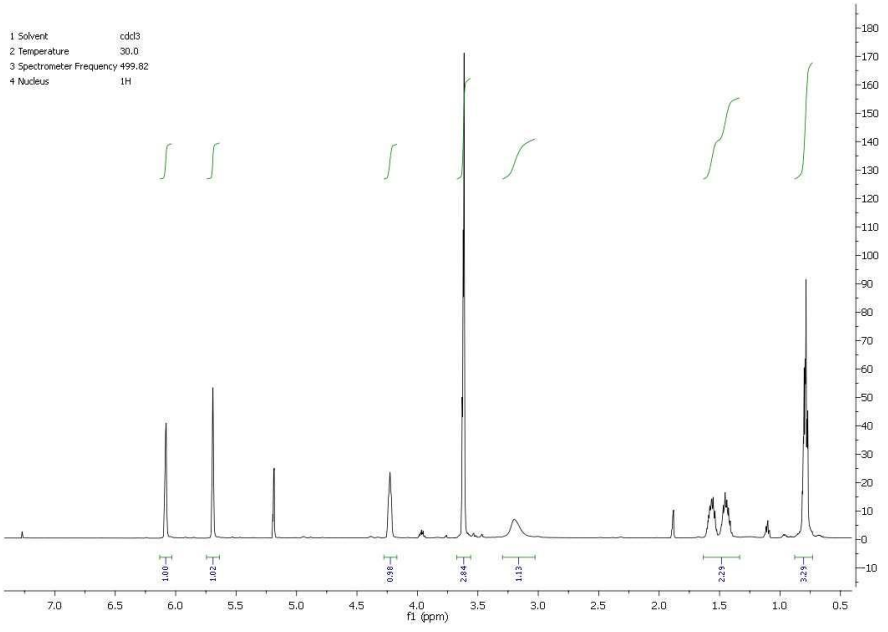
3 Spectrometer Frequency 499.82
4 Nucleus 1H
0.33
0.33
0.33
0.95
0.37
1.00
f1 (ppm)


167.06
143.58
123.98
76.93
67.18
51.83
22.12
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)


OH
O
OMe

**3.1b**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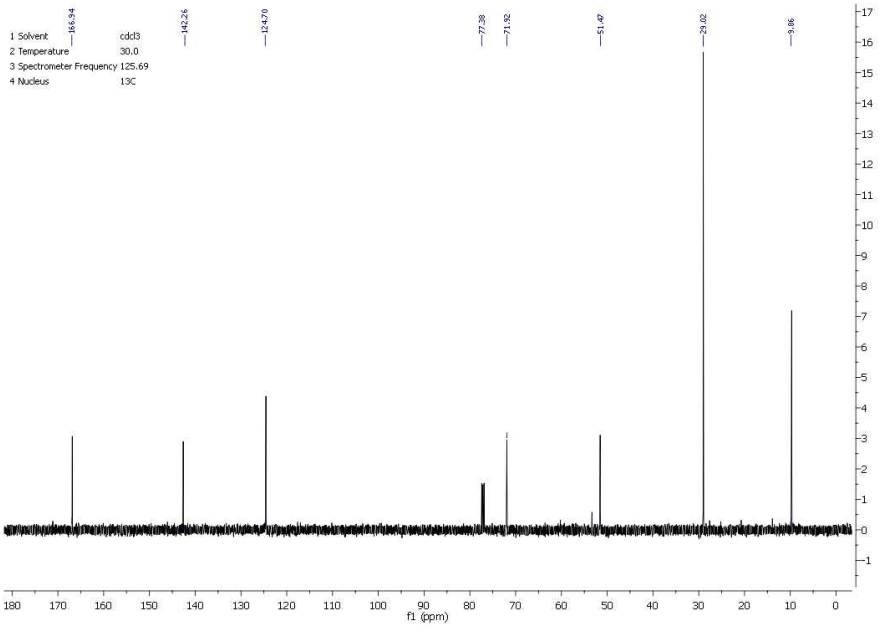
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
1.00
1.02
0.98
2.84
1.13
2.29
3.29
f1 (ppm)


166.94
142.26
124.70
77.39
71.92
51.47
29.02
9.86
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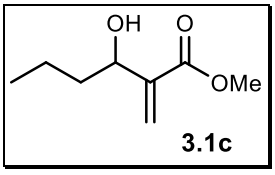
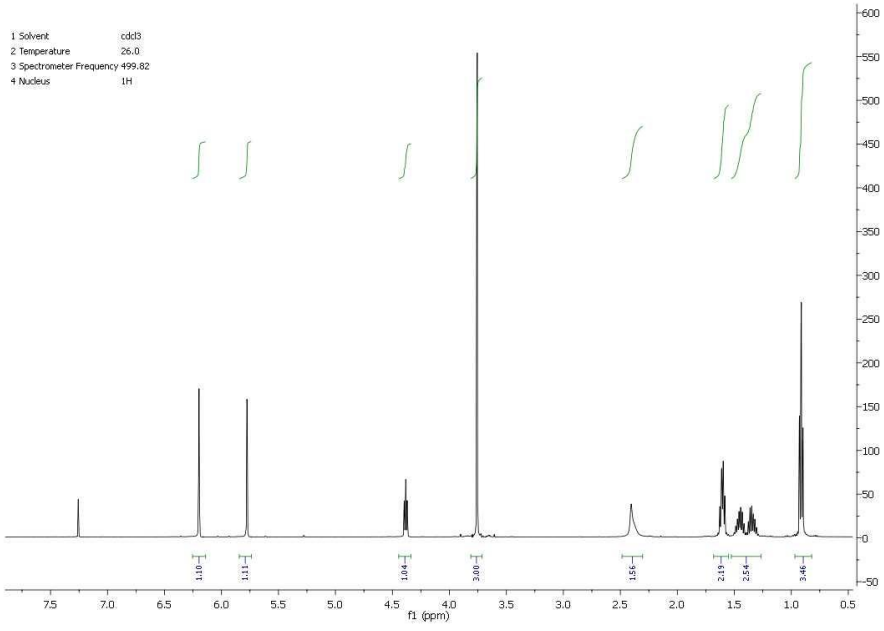
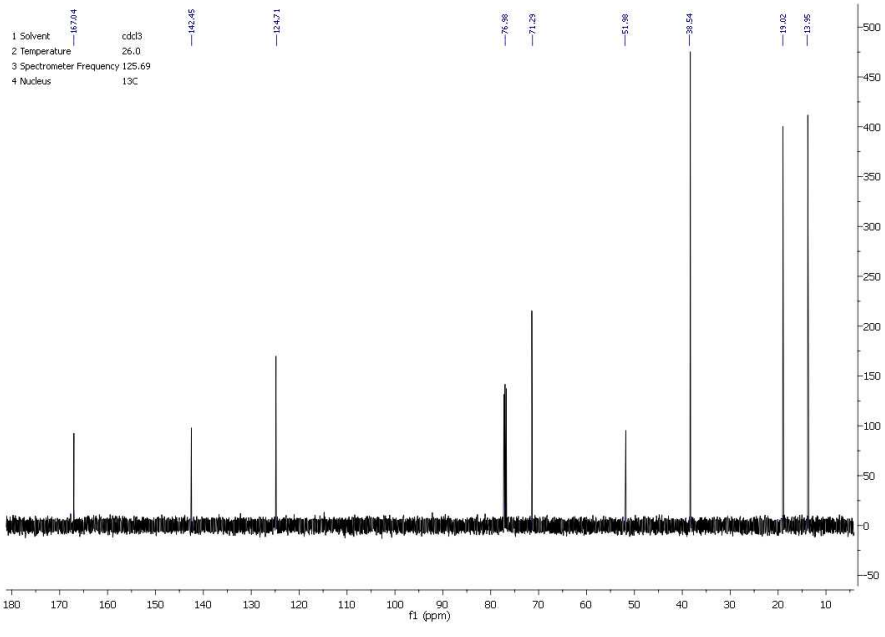
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)


OH
O
OMe
3.1c


1 Solvent cdcl3
2 Temperature 26.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
1.10
1.11
1.04
3.00
1.55
2.19
2.54
3.46
f1 (ppm)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2 Temperature 26.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
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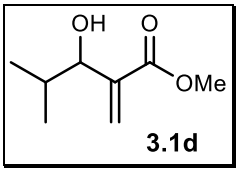
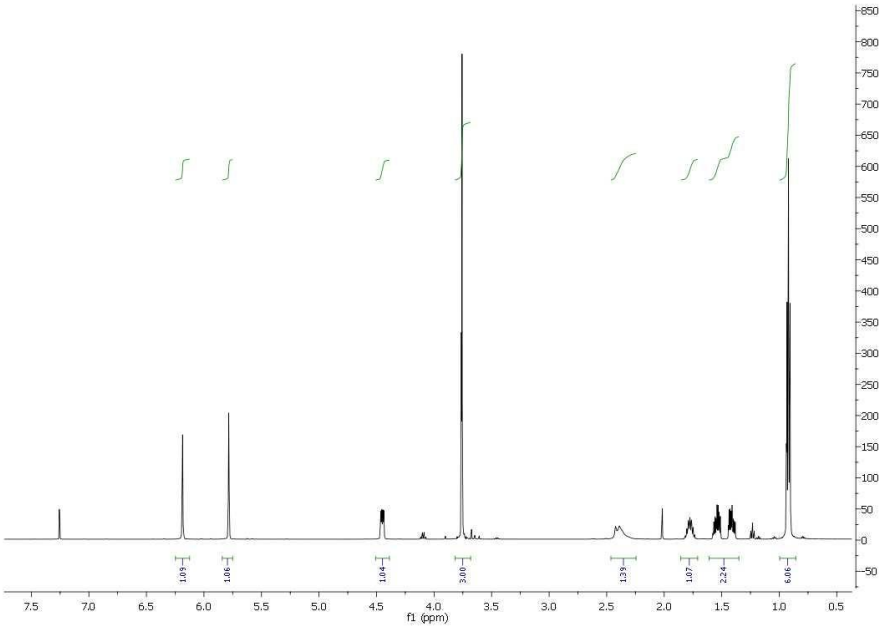
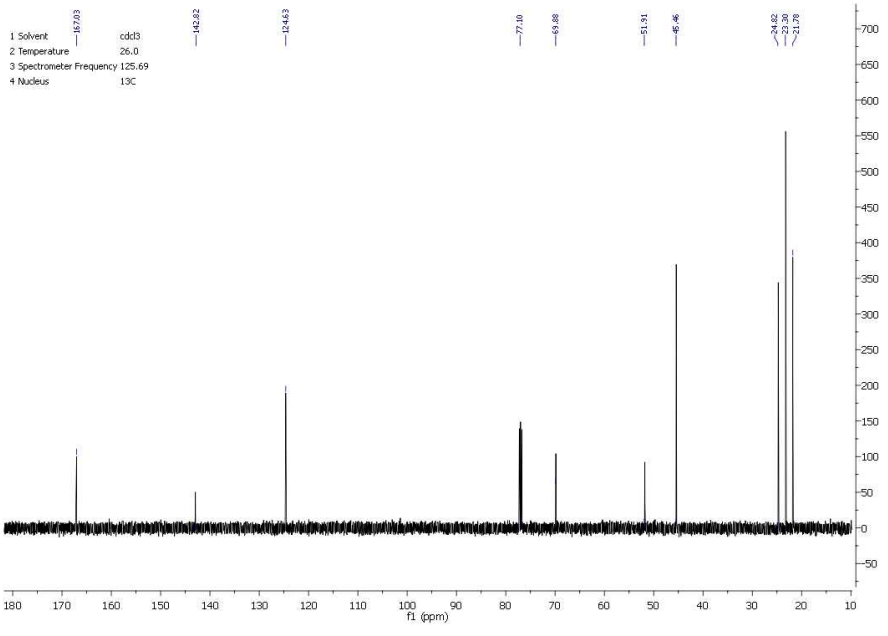
142.45
124.71
76.98
71.29
51.98
38.54
19.02
13.95
f1 (ppm)


OH
O
OMe
3.1d


1.09
1.06
1.04
3.00
1.39
1.07
2.24
6.06
f1 (ppm)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2 Temperature 26.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
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142.82
124.63
77.10
69.88
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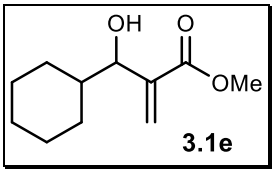
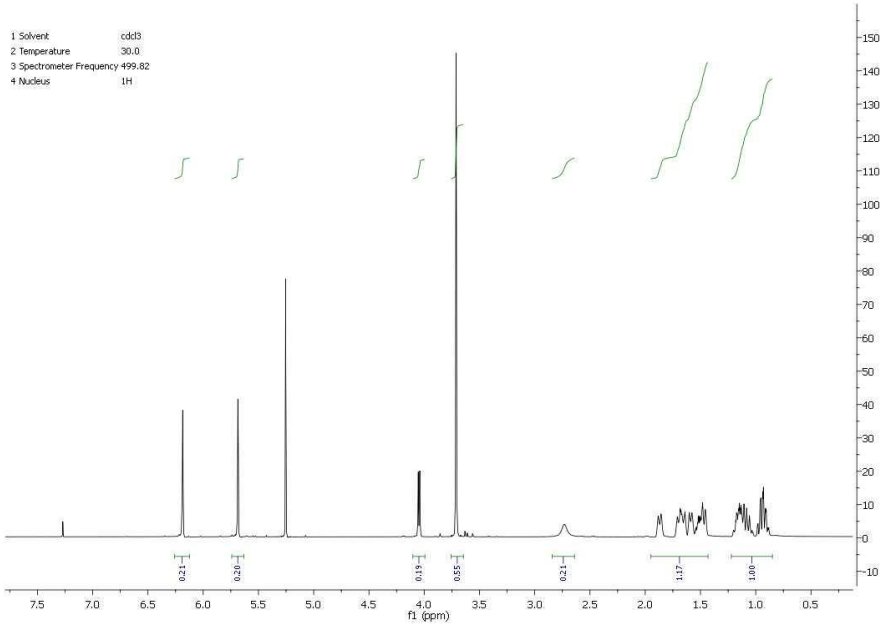
46.46
24.82
23.30
21.78
f1 (ppm)


OH
O
OMe
3.1e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3 Spectrometer Frequency 499.82
4 Nucleus 1H
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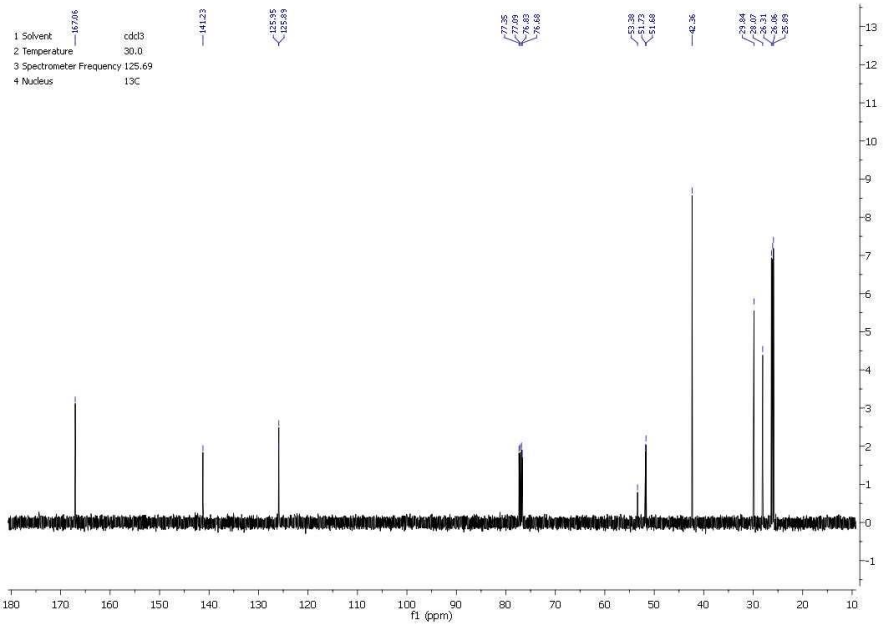
0.20
0.19
0.55
0.21
1.17
1.00
f1 (ppm)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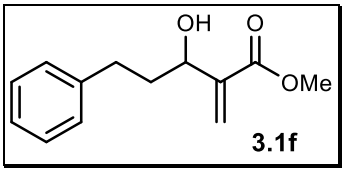
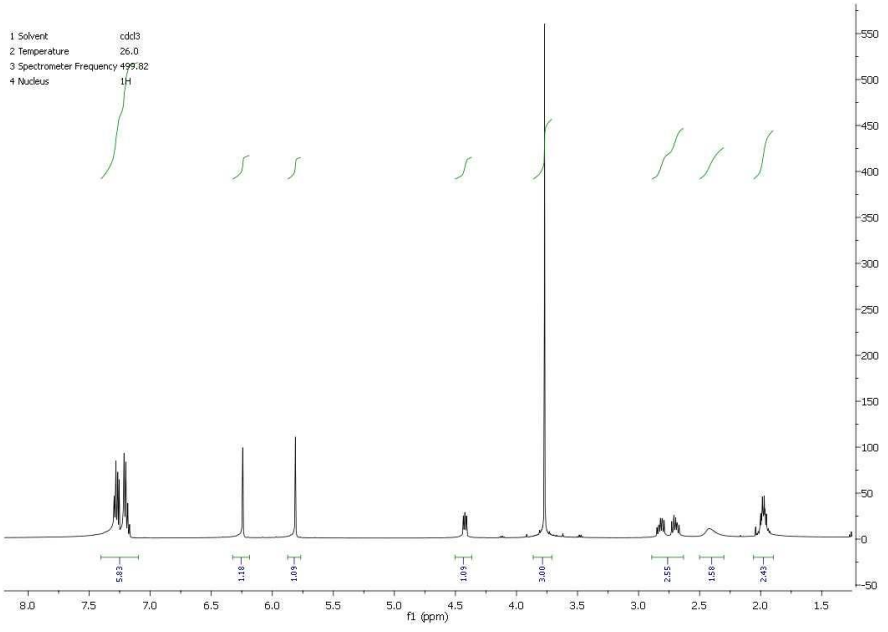
3 Spectrometer Frequency 125.69
4 Nucleus 13C
167.06
141.23
125.95
125.83
77.35
77.09
76.83
76.68
53.38
51.73
51.68
42.36
29.84
29.07
26.31
26.06
25.60
f1 (ppm)


OH
O
OMe
3.1f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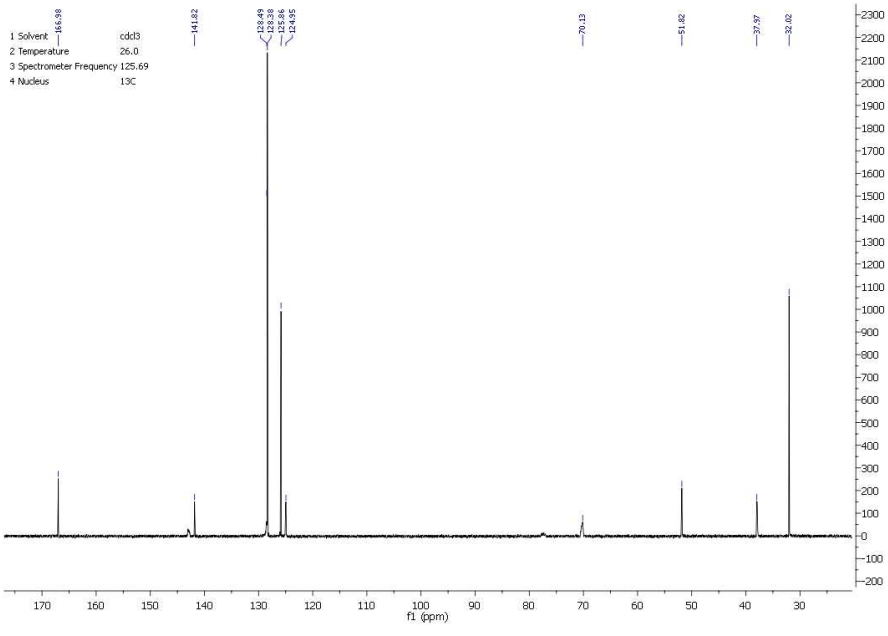
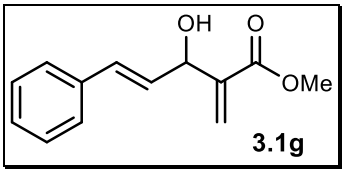
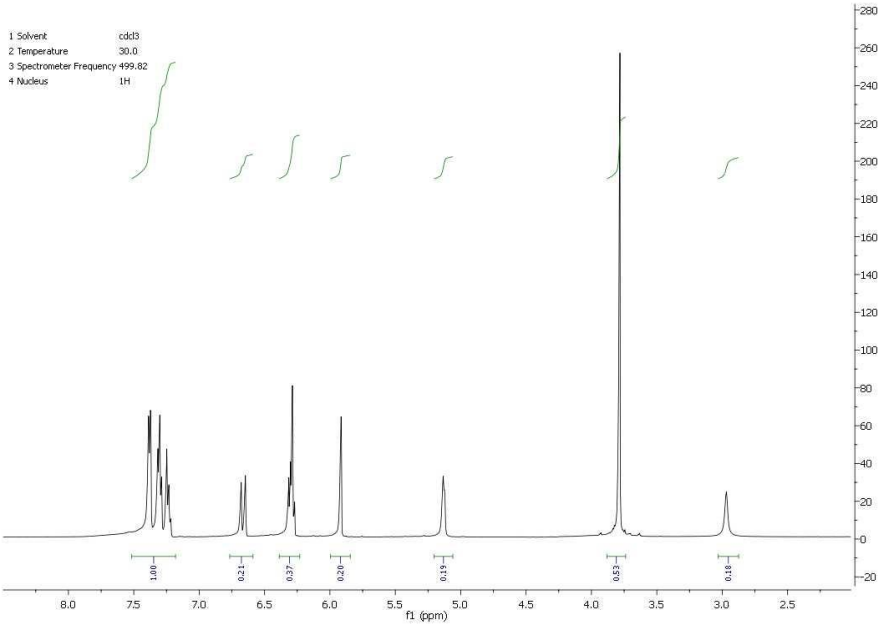
2 Temperature 26.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
5.83
1.18
1.09
1.09
3.00
2.55
1.58
2.43
f1 (ppm)


166.98
141.82
128.49
128.38
125.86
124.95
70.13
51.82
37.97
32.02
1 Solvent cdcl3
2 Temperature 26.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)


OH
O
OMe
3.1g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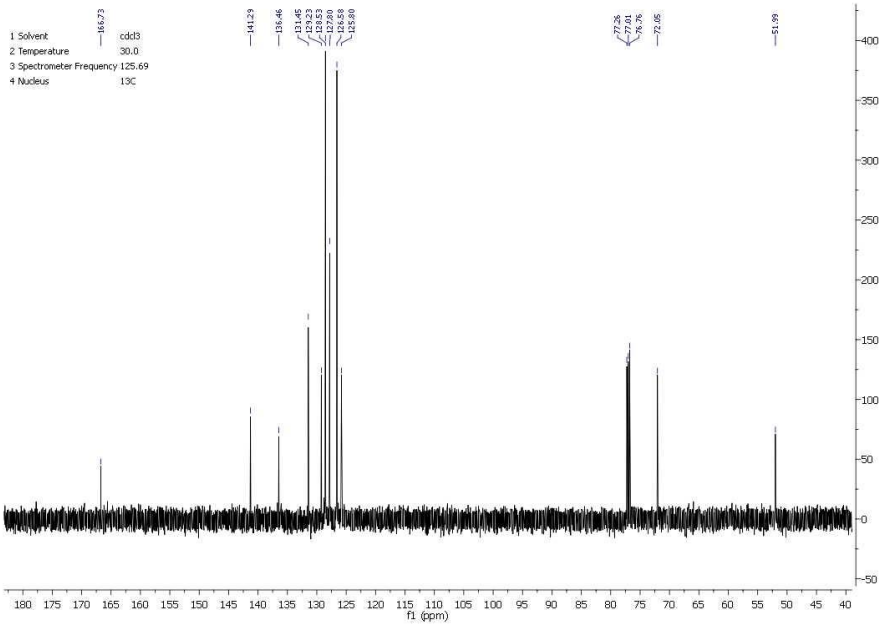
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
1.00
0.21
0.37
0.20
0.19
0.83
0.18
f1 (ppm)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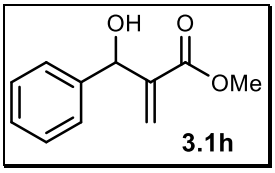
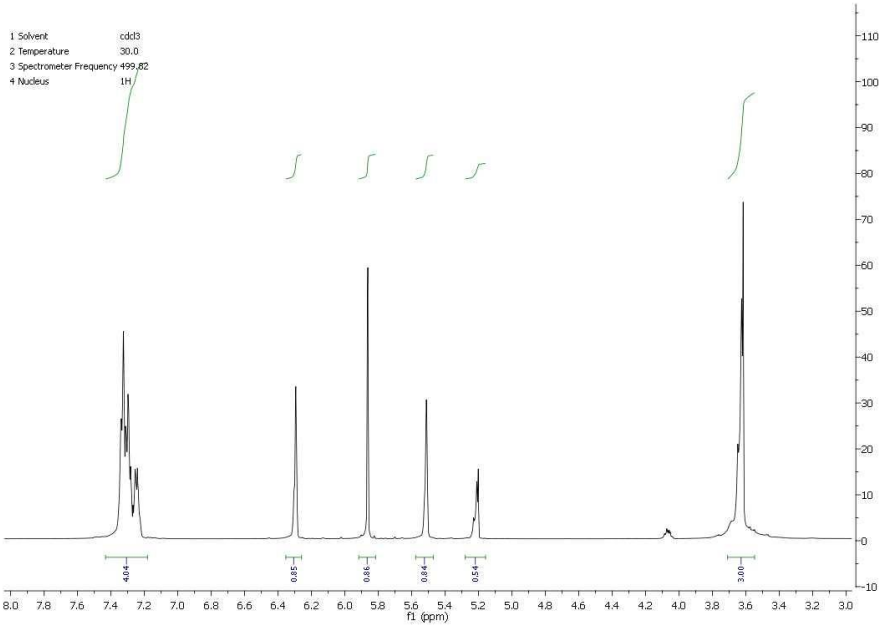
3 Spectrometer Frequency 125.69
4 Nucleus 13C
166.73
141.29
136.46
131.46
129.23
128.53
127.80
126.58
125.80
77.26
77.01
76.76
72.05
51.99
f1 (ppm)


OH
O
OMe
3.1h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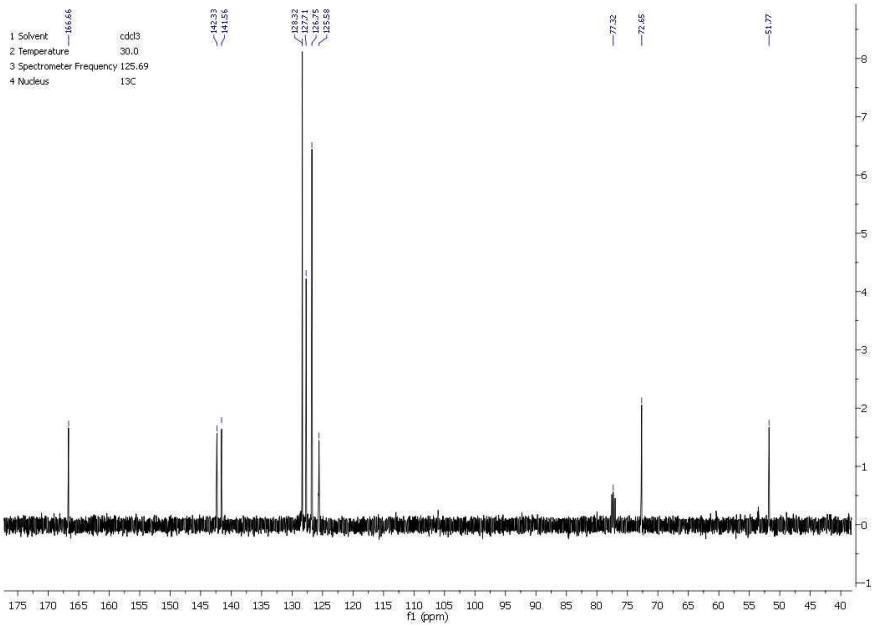
0.85
0.86
0.84
0.54
3.00
f1 (ppm)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3 Spectrometer Frequency 125.69
4 Nucleus 13C
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141.56
128.32
127.71
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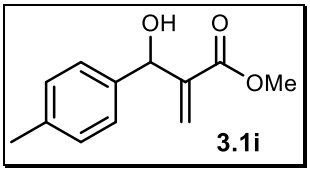
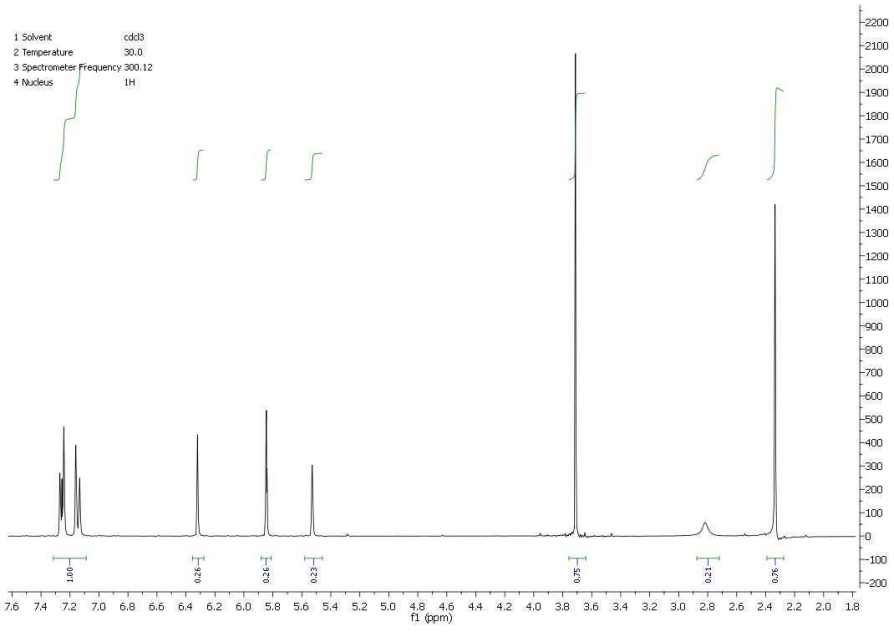
125.58
77.32
72.65
51.77
f1 (ppm)


OH
O
OMe
3.1i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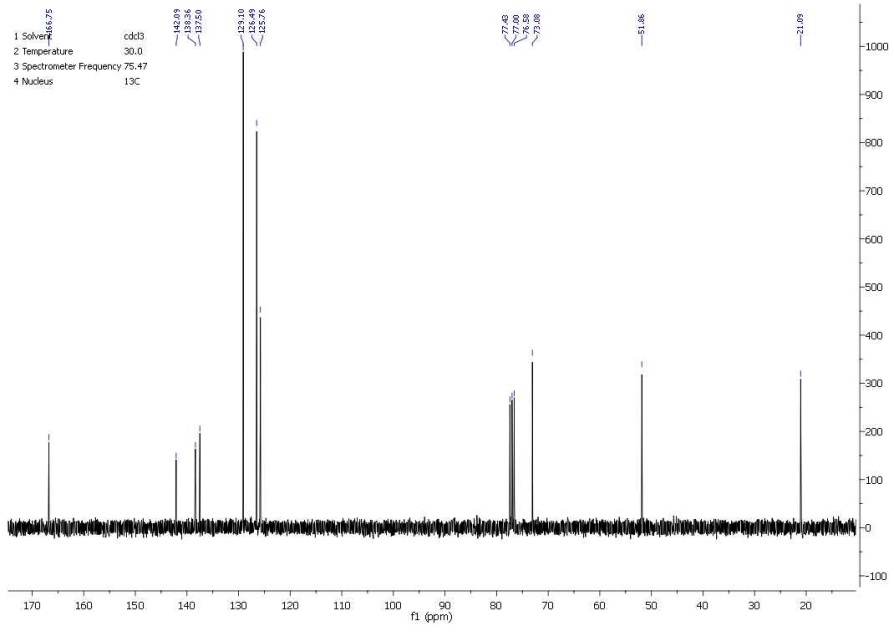
4 Nucleus 1H
f1 (ppm)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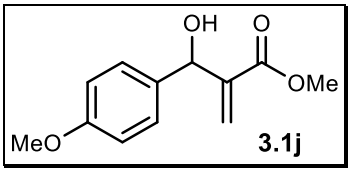
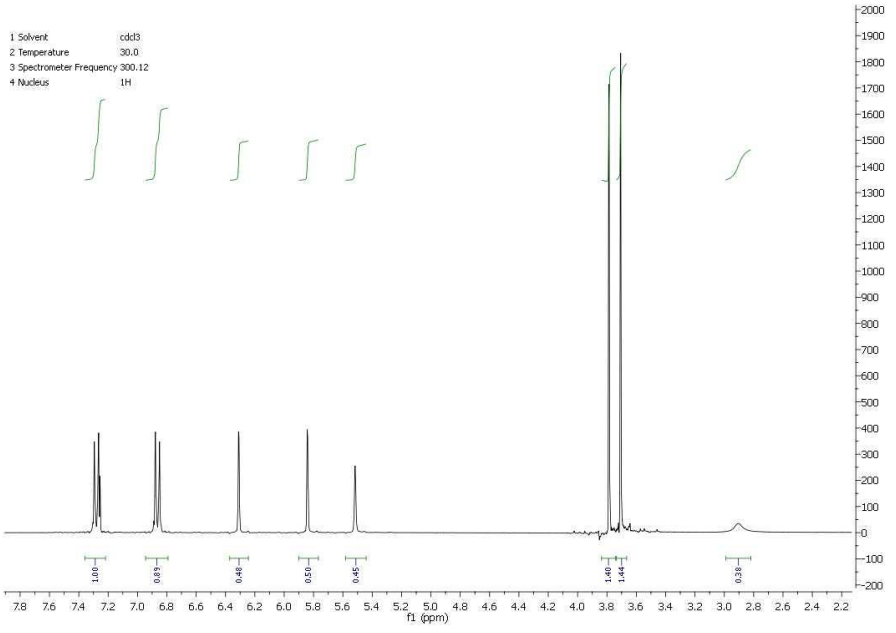
3 Spectrometer Frequency 75.47
4 Nucleus 13C
f1 (ppm)


OH
O
OMe
MeO
3.1j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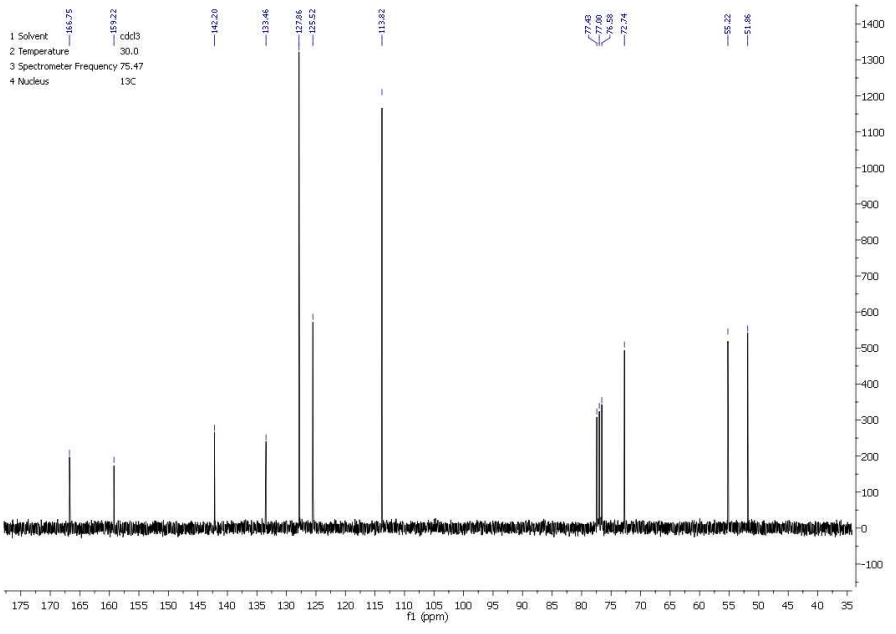
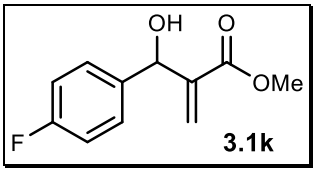
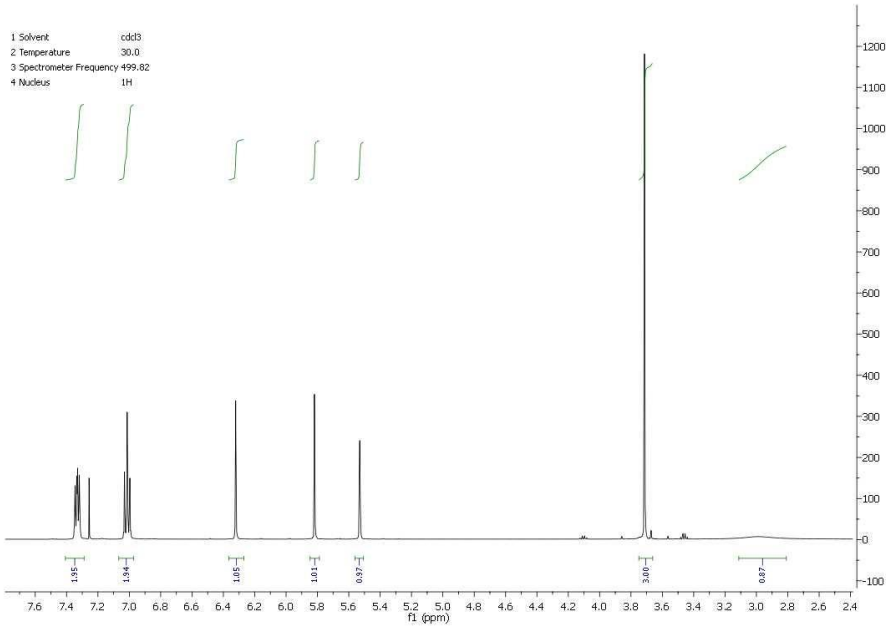
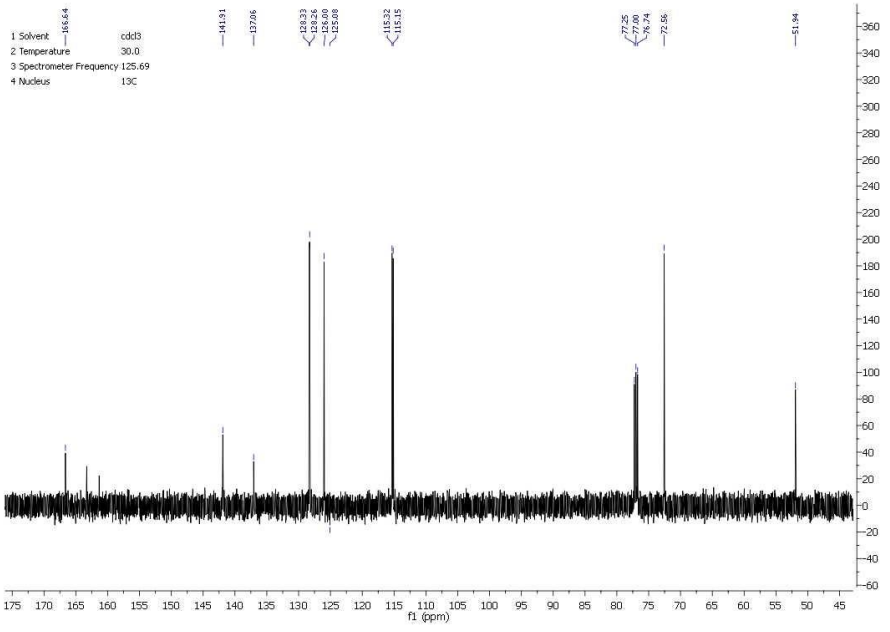
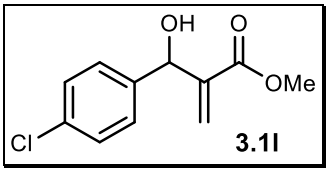
3 Spectrometer Frequency 300.12
4 Nucleus 1H
1.00
0.89
0.48
0.50
0.45
1.40
1.44
0.38
f1 (ppm)


1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 75.47
4 Nucleus 13C
166.75
159.22
142.20
133.46
127.86
125.52
113.82
77.40
77.00
76.58
72.74
55.22
51.86
f1 (ppm)

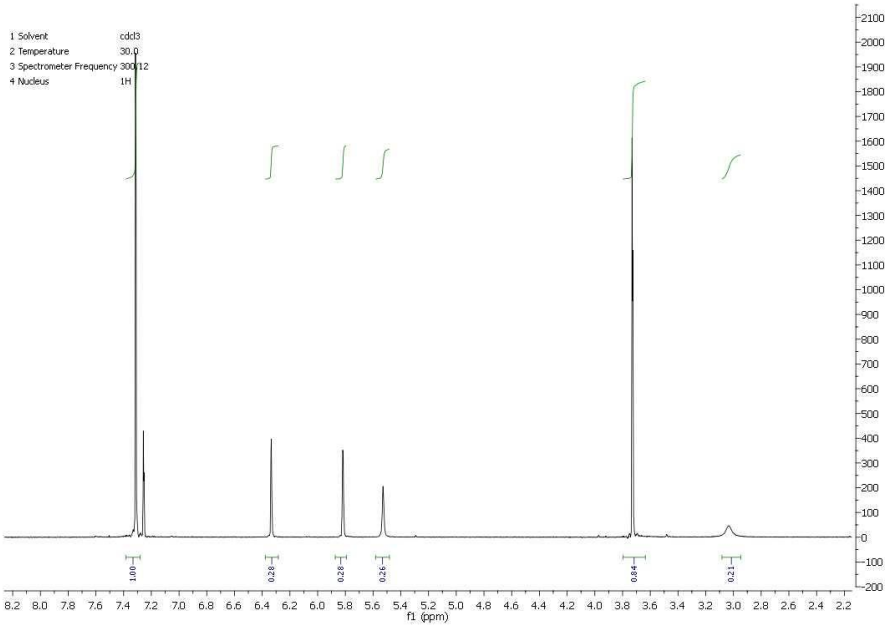

OH
O
OMe
F
3.1k

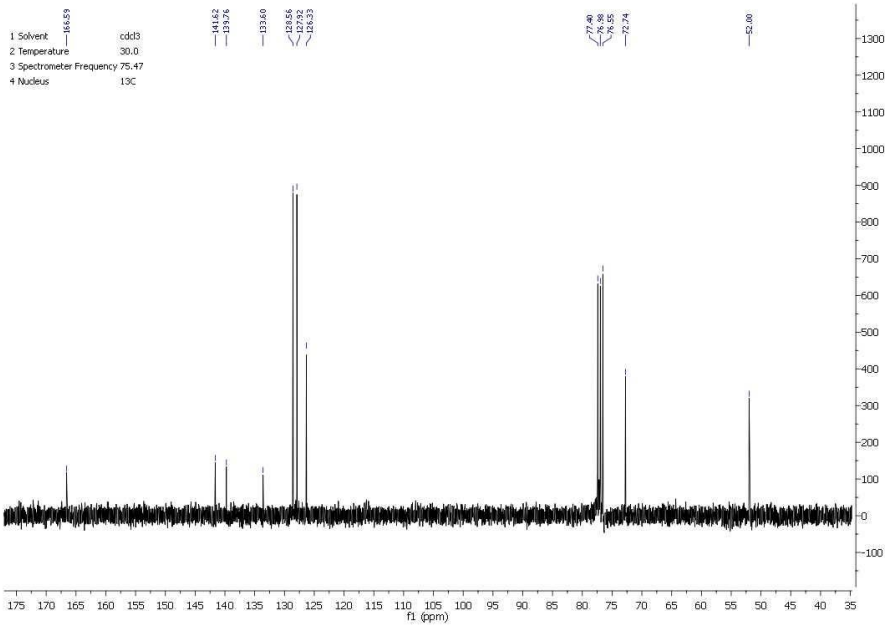

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
1.95
1.94
1.05
1.01
0.97
3.00
0.87
f1 (ppm)

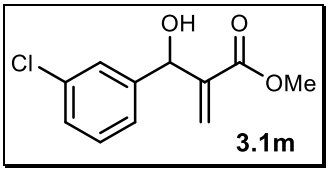

166.64
141.91
137.06
129.33
129.26
125.00
125.00
115.32
115.15
77.25
77.00
76.74
72.56
51.94
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

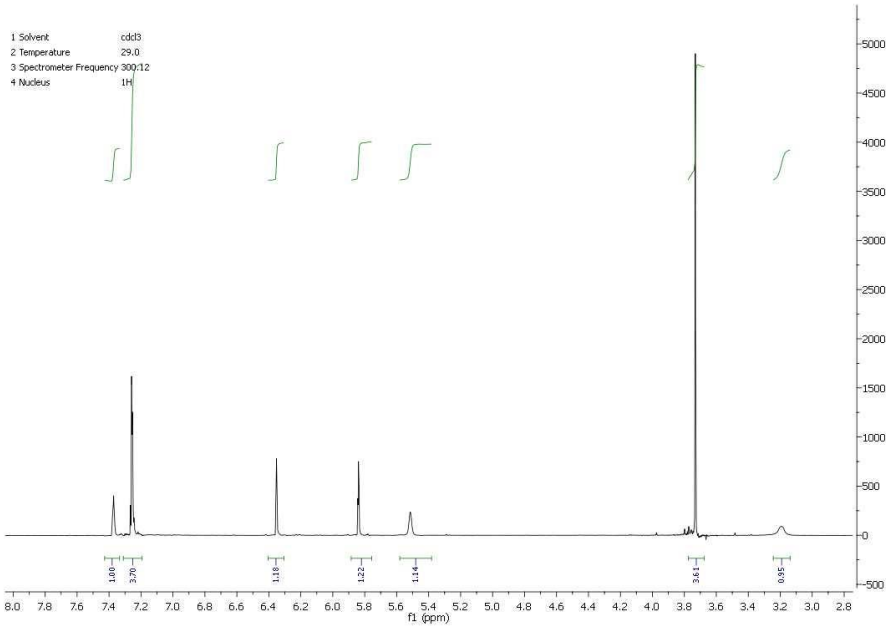

OH
O
OMe
Cl

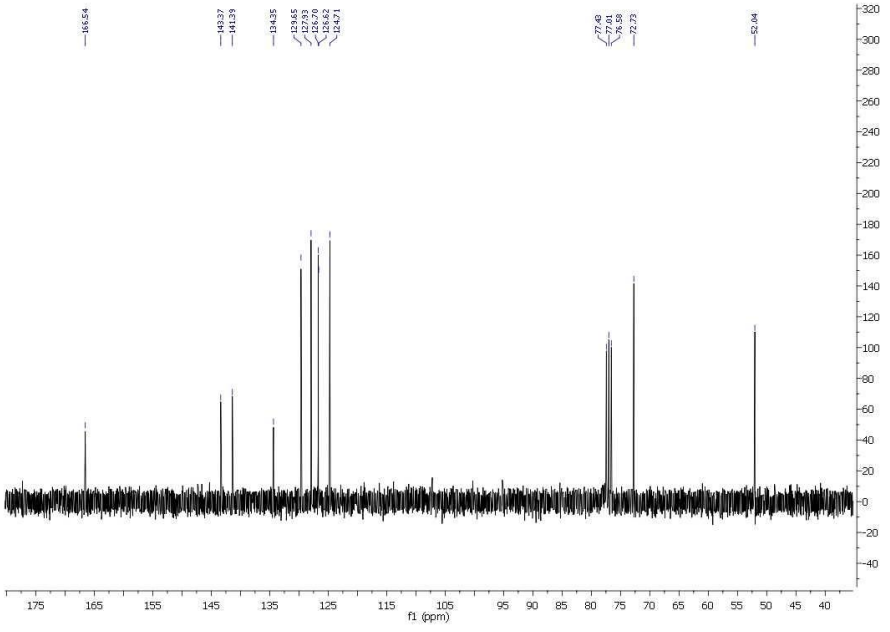
**3.1l**

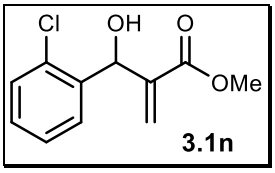

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 300.12
4 Nucleus 1H
1.00
0.28
0.28
0.26
0.84
0.21
f1 (ppm)

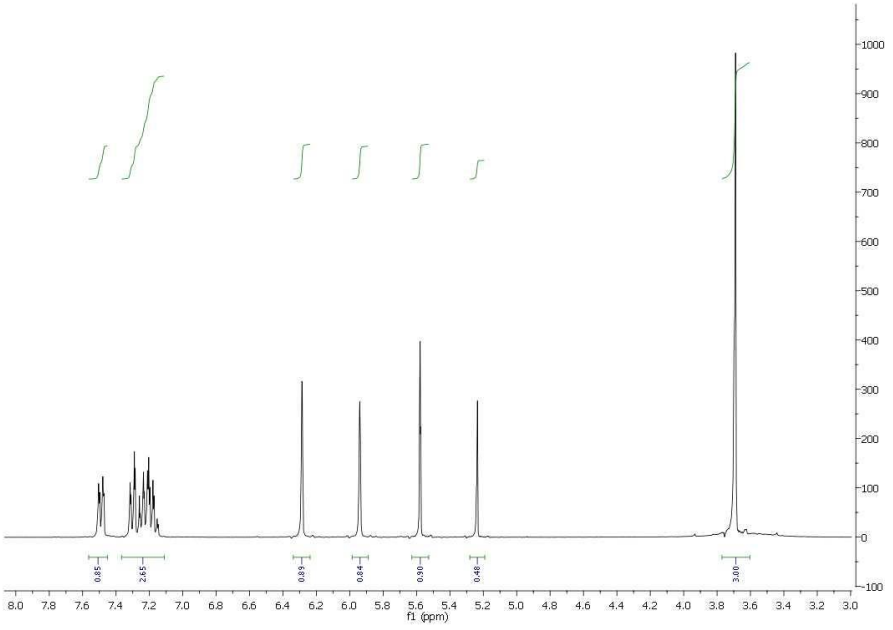

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 75.47
4 Nucleus 13C
166.89
141.62
139.76
133.60
128.56
127.92
126.33
77.40
76.98
76.55
72.74
52.00
f1 (ppm)

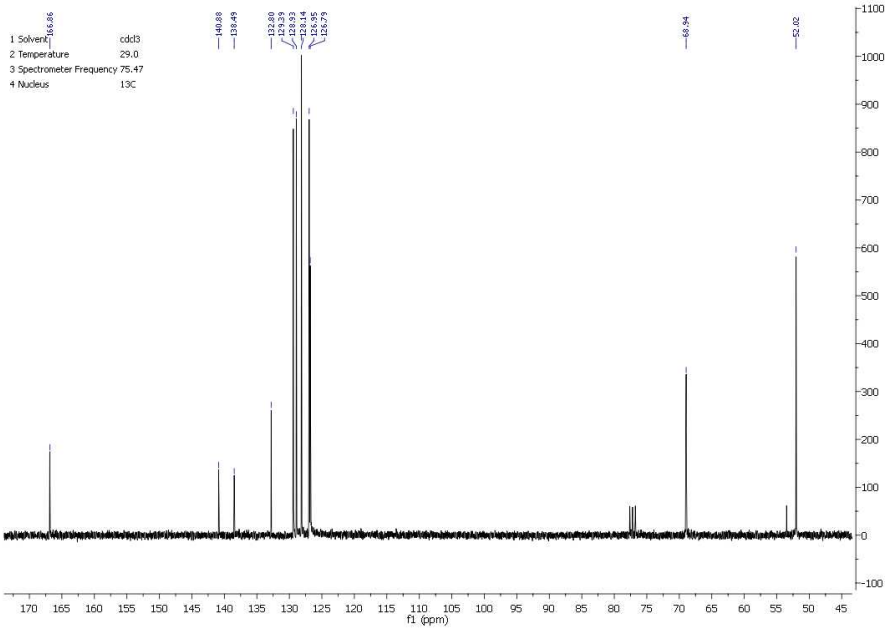

OH
O
Cl
OMe
3.1m

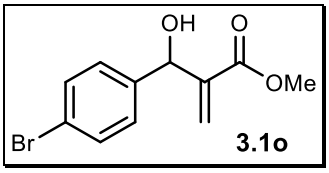

1 Solvent cdcl3
2 Temperature 29.0
3 Spectrometer Frequency 300.12
4 Nucleus 1H
1.00
3.70
1.18
1.22
1.14
3.61
0.95
f1 (ppm)

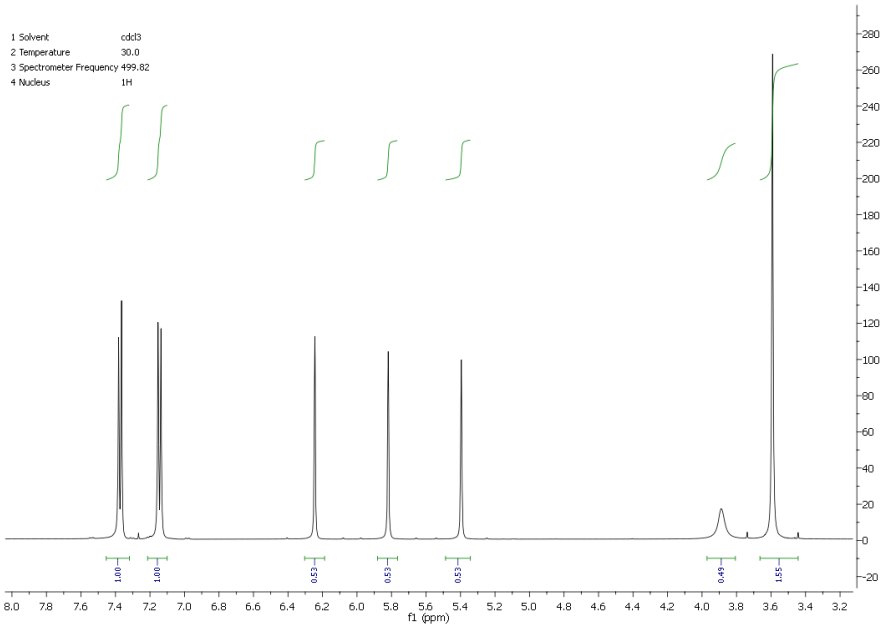

166.54
143.37
141.39
134.35
129.65
127.93
126.70
126.62
124.71
77.43
77.01
76.59
72.73
52.04
f1 (ppm)

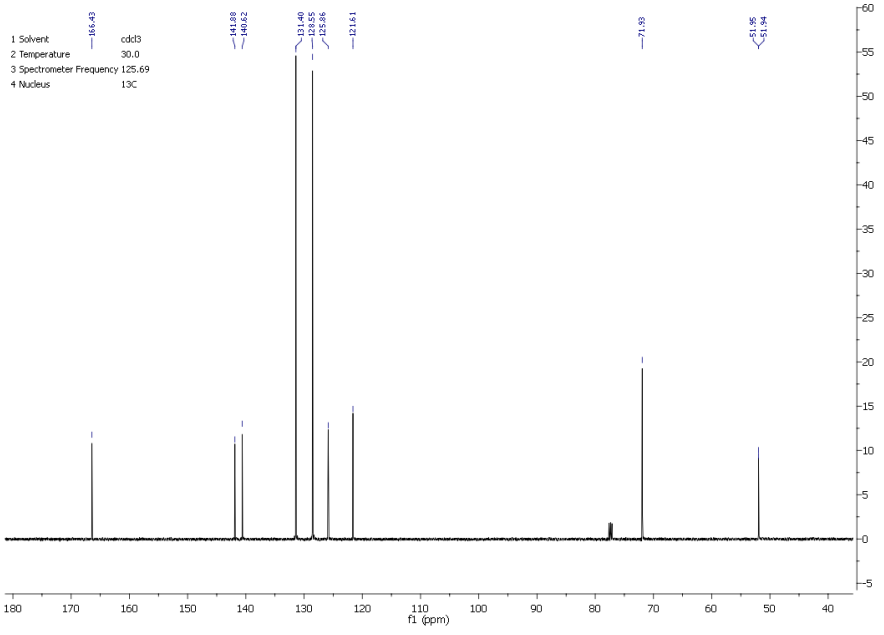

Cl
OH
O
OMe
3.1n

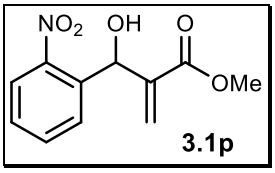

0.85
2.65
0.89
0.84
0.90
0.48
3.00
f1 (ppm)

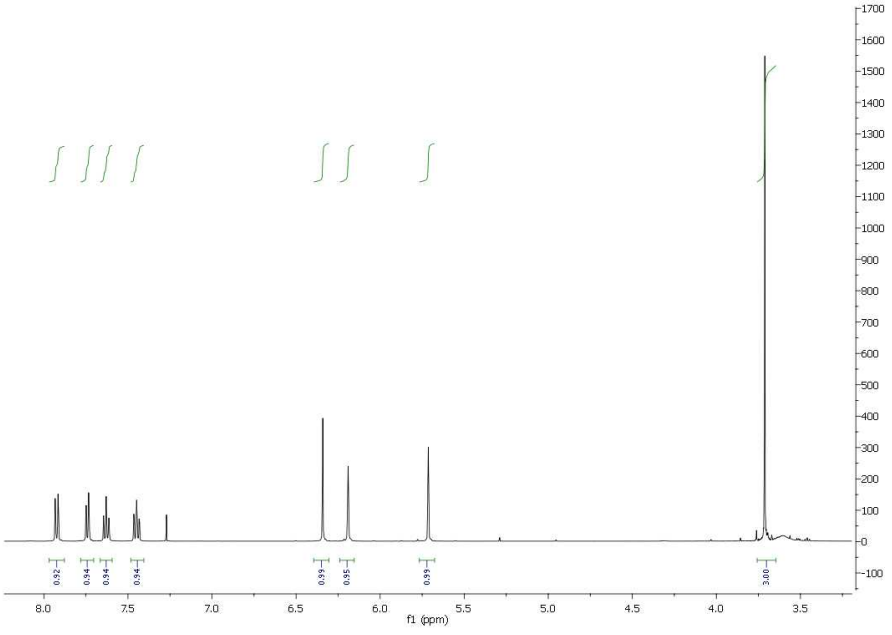

166.86
140.88
138.49
132.80
129.39
128.93
128.14
126.95
126.73
68.94
52.02
1 Solvent cdcl3
2 Temperature 29.0
3 Spectrometer Frequency 75.47
4 Nucleus 13C
f1 (ppm)

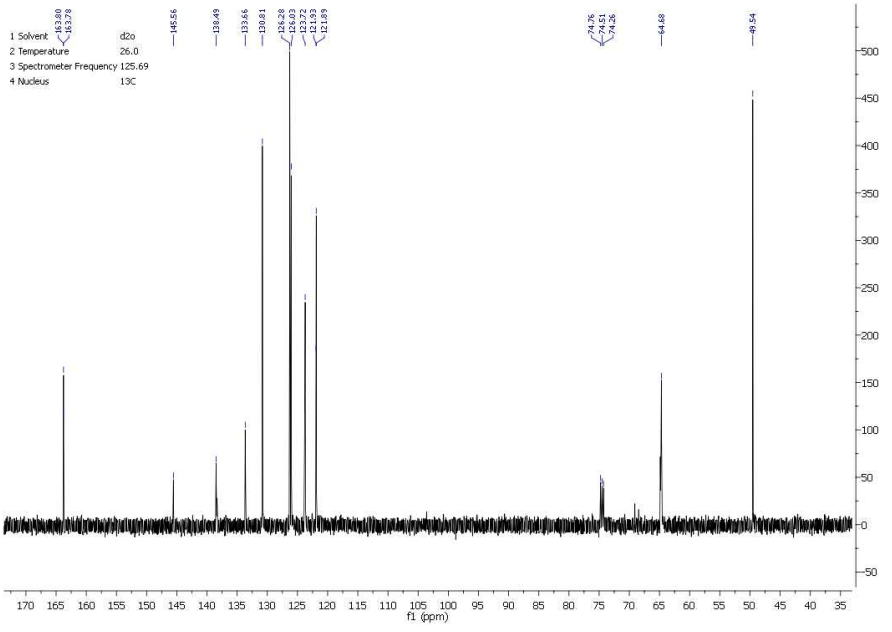

OH
O
OMe
Br
3.1o

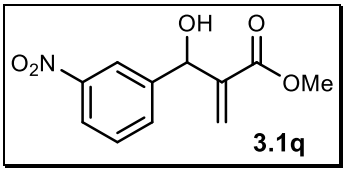

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

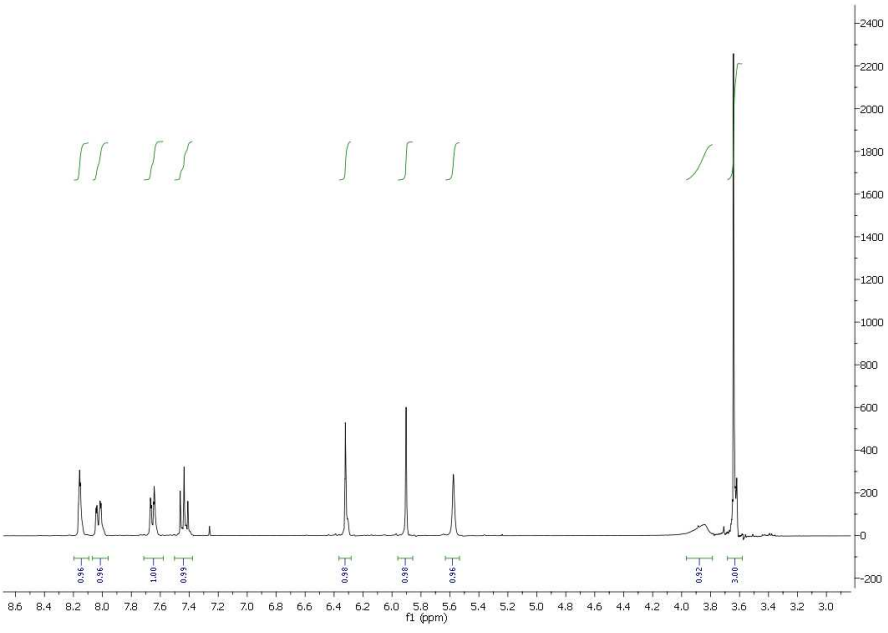

166.43
141.88
140.62
131.40
128.55
125.86
121.61
71.93
51.95
51.94
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

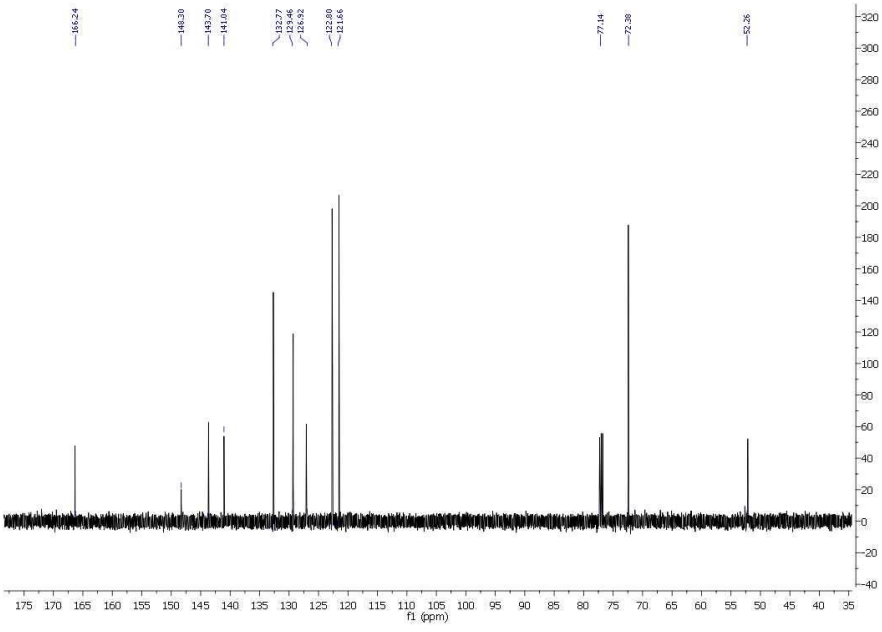

NO2
OH
O
OMe
3.1p

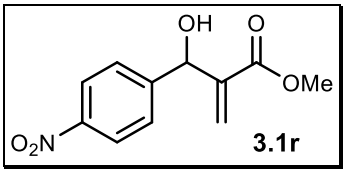

0.92
0.94
0.94
0.94
0.99
0.95
0.99
3.00
f1 (ppm)

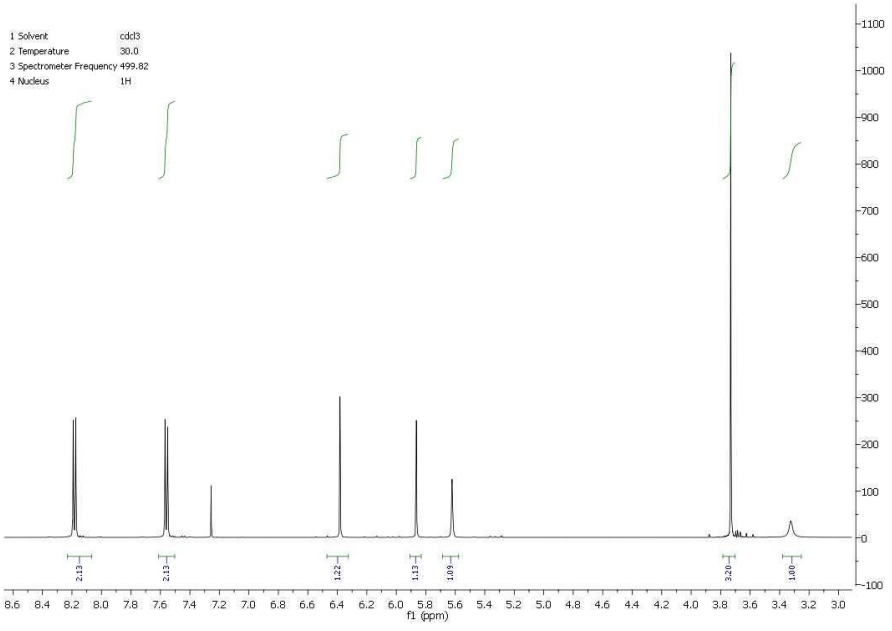

1 Solvent d2o
2 Temperature 26.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
163.80
163.78
145.56
138.49
133.66
130.81
126.28
126.03
125.73
121.93
121.89
74.76
74.51
74.26
64.68
49.54
f1 (ppm)

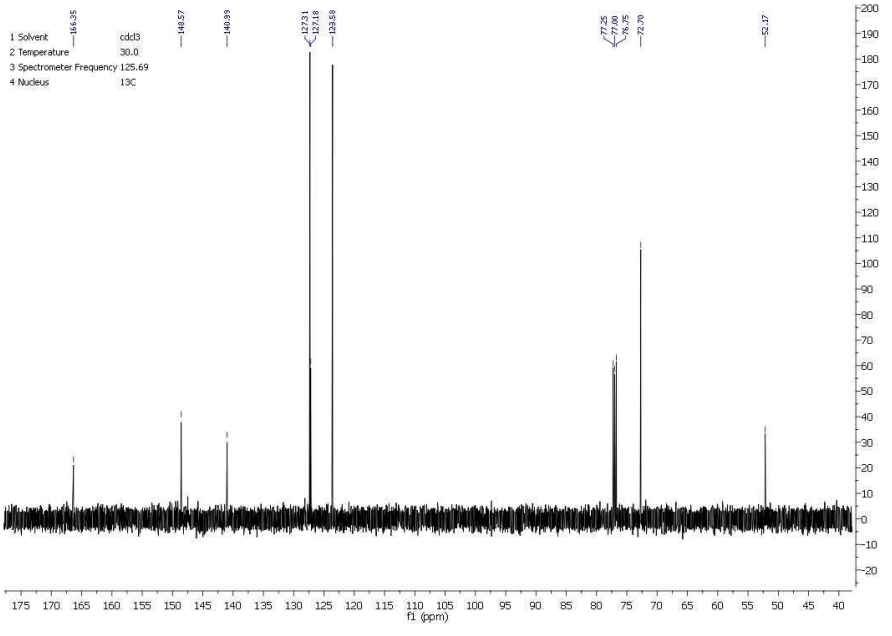

OH
O
$O_2N$
OMe
3.1q

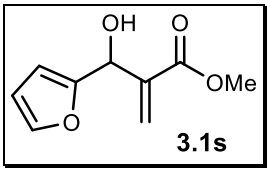

0.56
0.96
1.00
0.99
0.98
0.98
0.96
0.92
3.00
f1 (ppm)

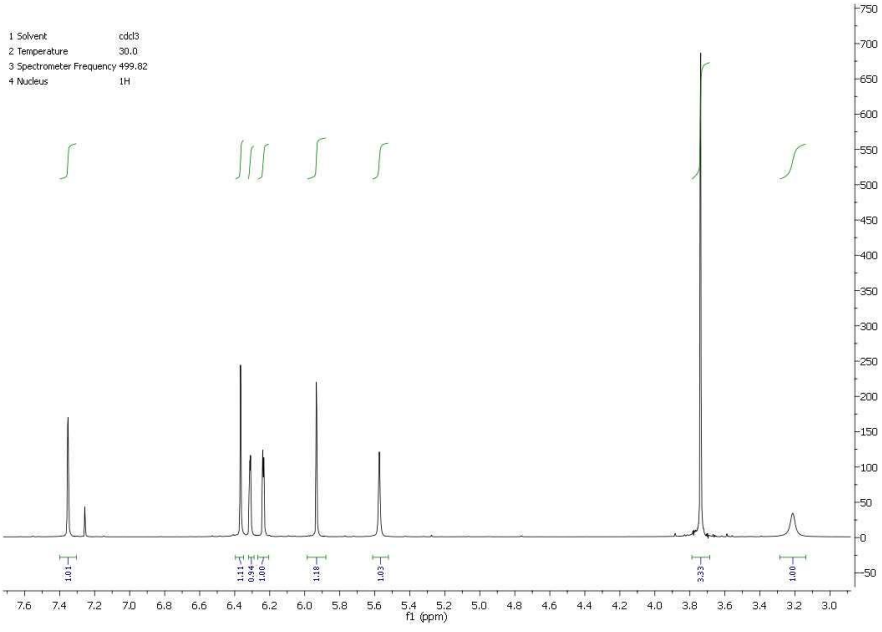

166.24
149.30
143.70
141.04
133.77
129.46
126.92
122.80
121.66
77.14
72.38
52.26
f1 (ppm)

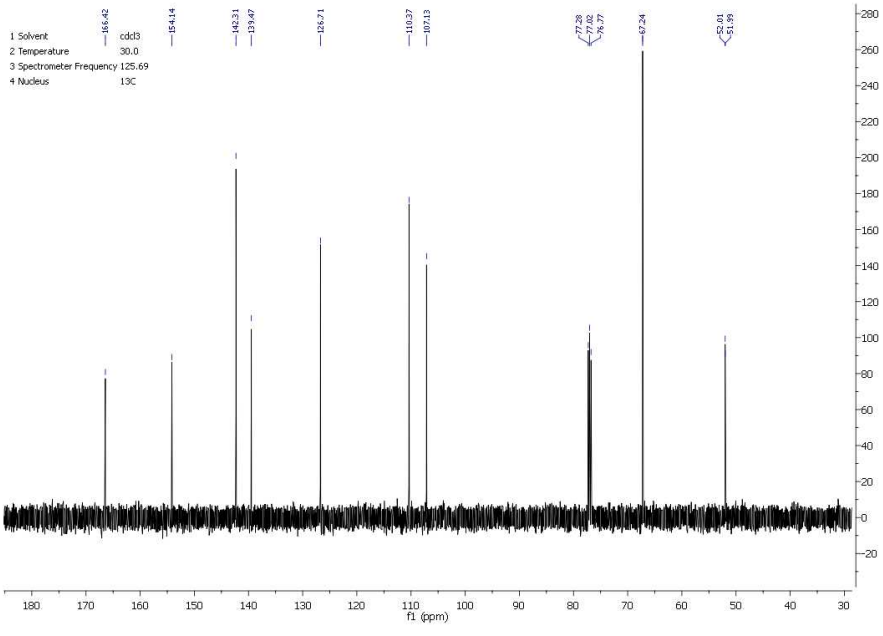

OH
O
OMe
O2N
3.1r

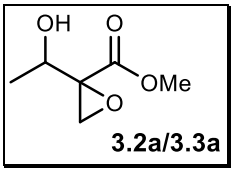

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

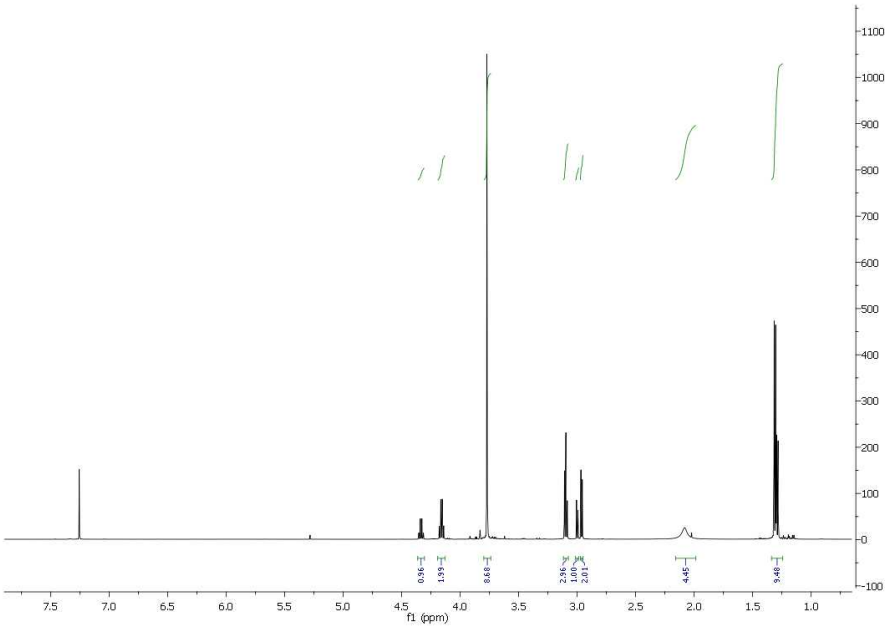

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
164.35
149.57
140.99
127.31
127.16
125.58
77.25
77.00
76.75
72.70
52.17
f1 (ppm)

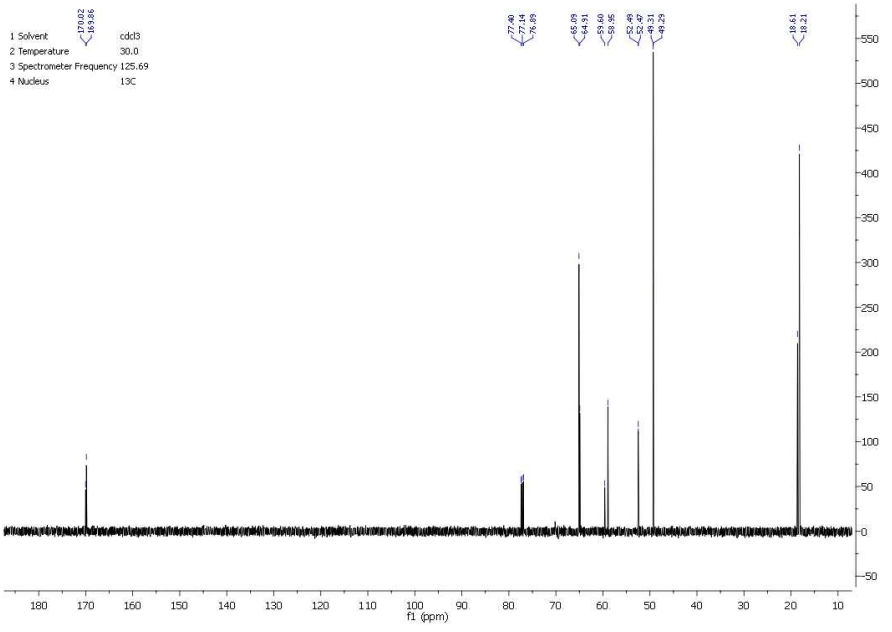

OH
O
OMe
O
3.1s

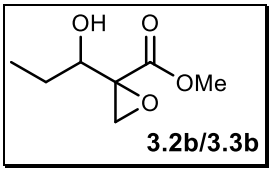

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

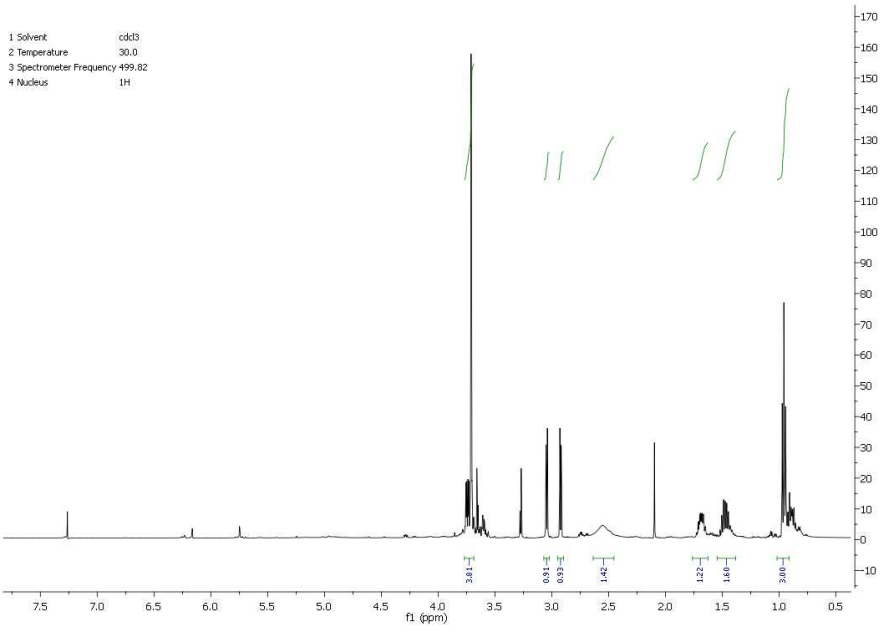

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
166.42
154.14
142.31
139.47
126.71
110.37
107.13
77.28
77.02
76.77
67.24
52.01
51.99
f1 (ppm)

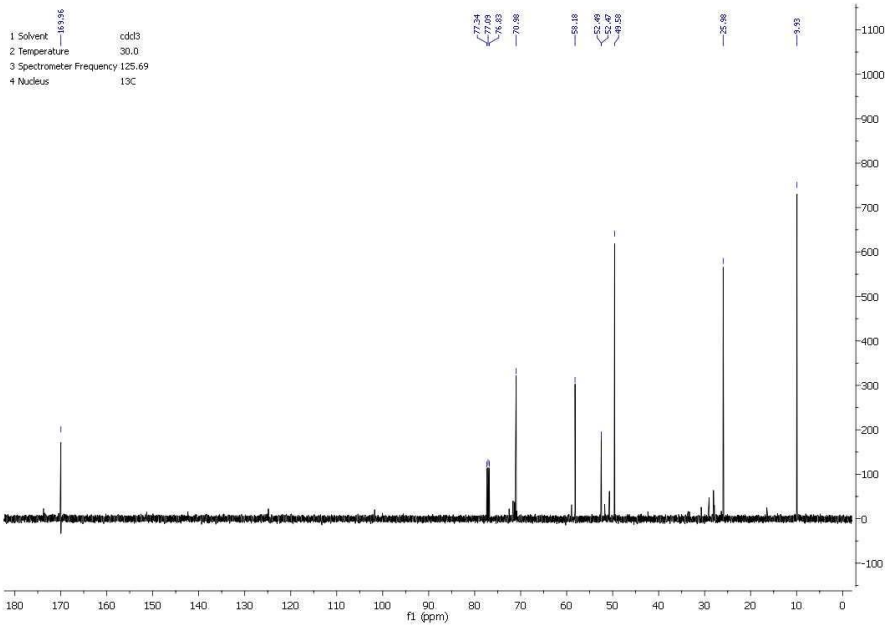

OH
O
OMe
O
3.2a/3.3a

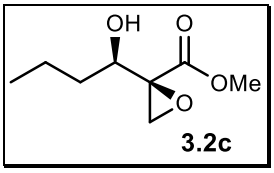

f1 (ppm)


1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

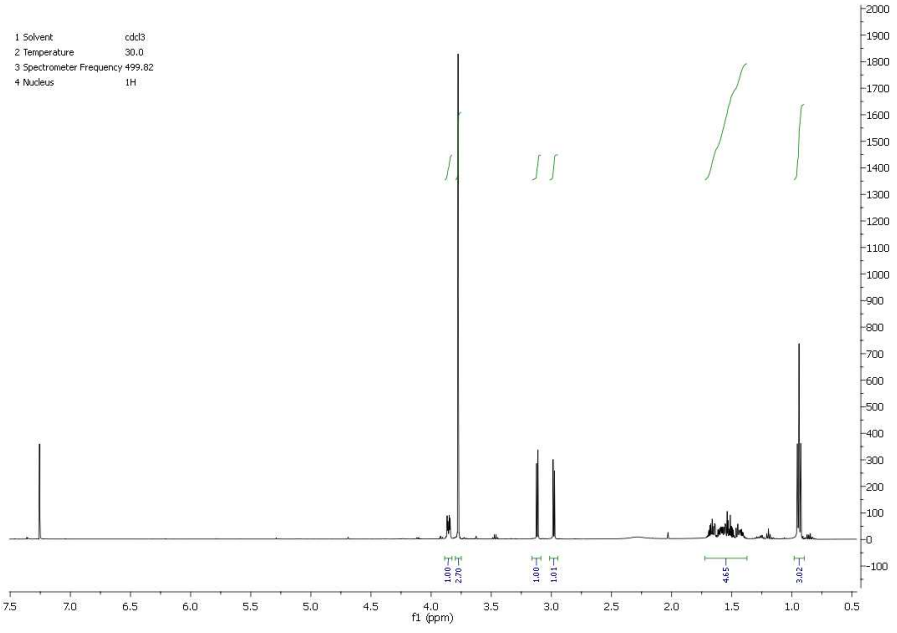

OH
O
OMe
O
3.2b/3.3b

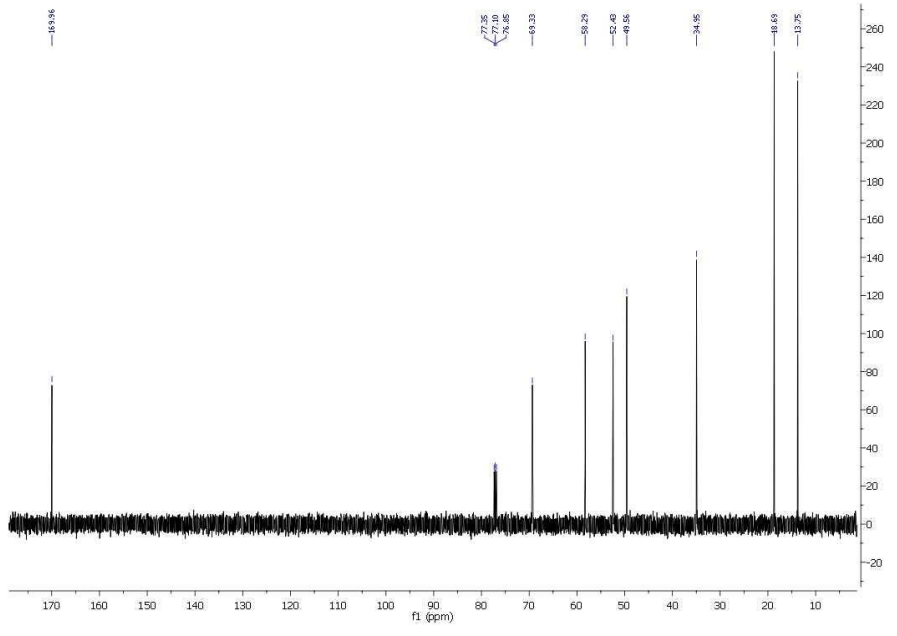

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
3.81
0.91
0.93
1.42
1.22
1.60
3.00
f1 (ppm)

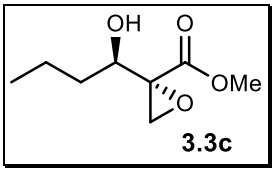

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
169.56
77.54
77.05
76.83
70.98
58.18
52.49
52.47
49.59
25.98
9.93
f1 (ppm)


OH
O
OMe
O

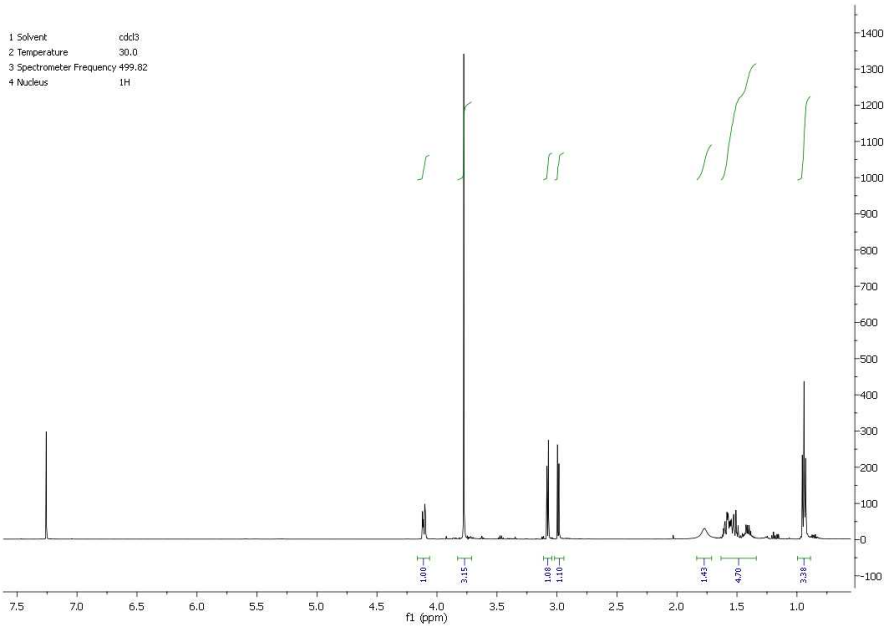
**3.2c**

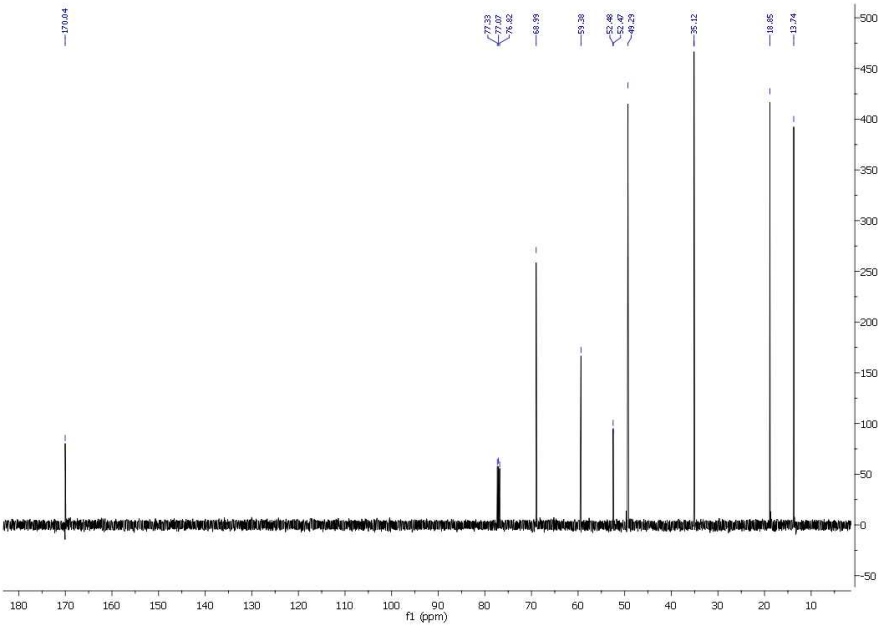

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

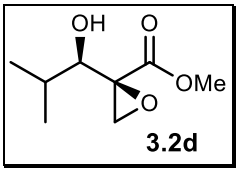

169.96
77.35
77.10
76.85
69.33
58.29
52.49
49.56
34.56
18.69
13.75
f1 (ppm)

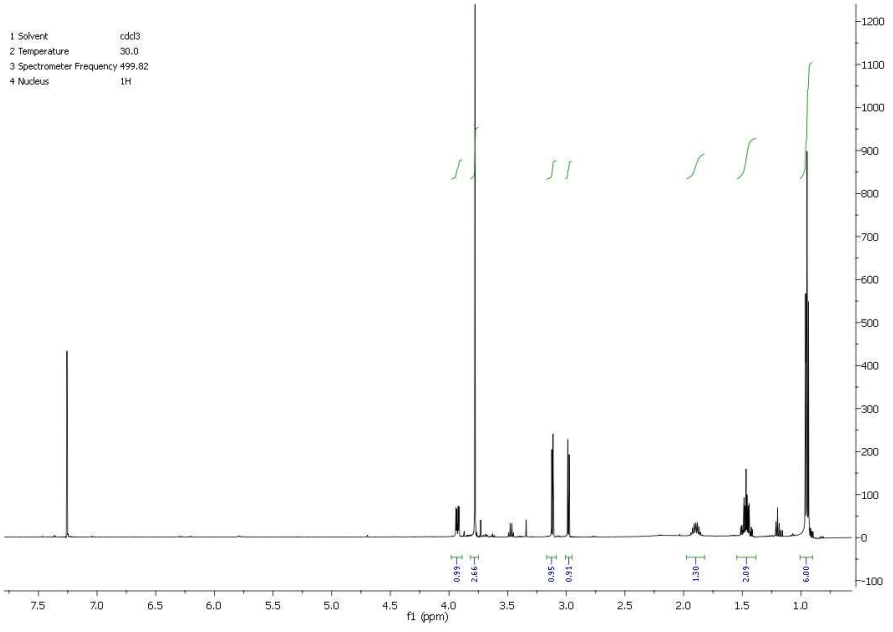

OH
O
OMe
O

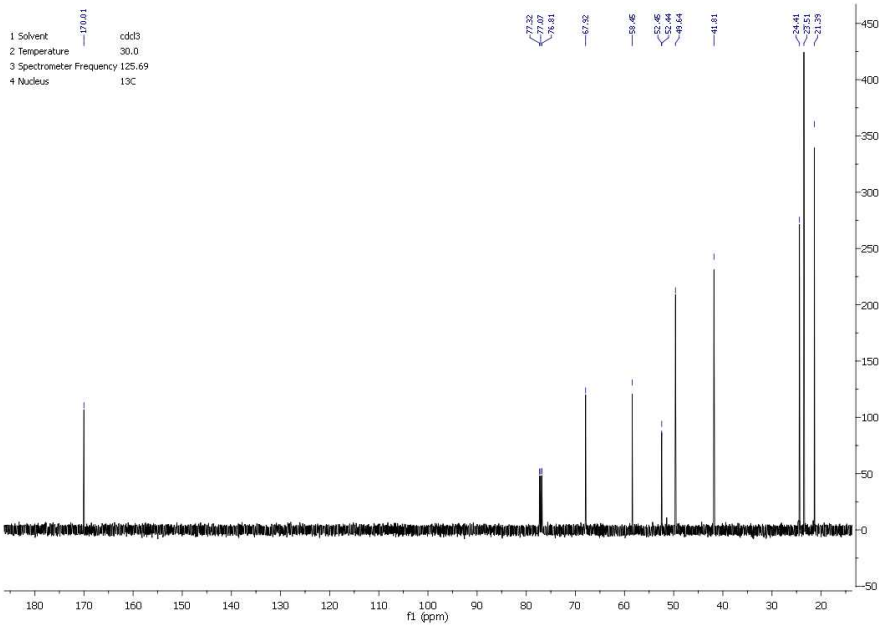
**3.3c**

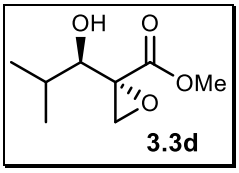

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
1.00
3.15
1.08
1.10
1.43
4.70
3.38
f1 (ppm)

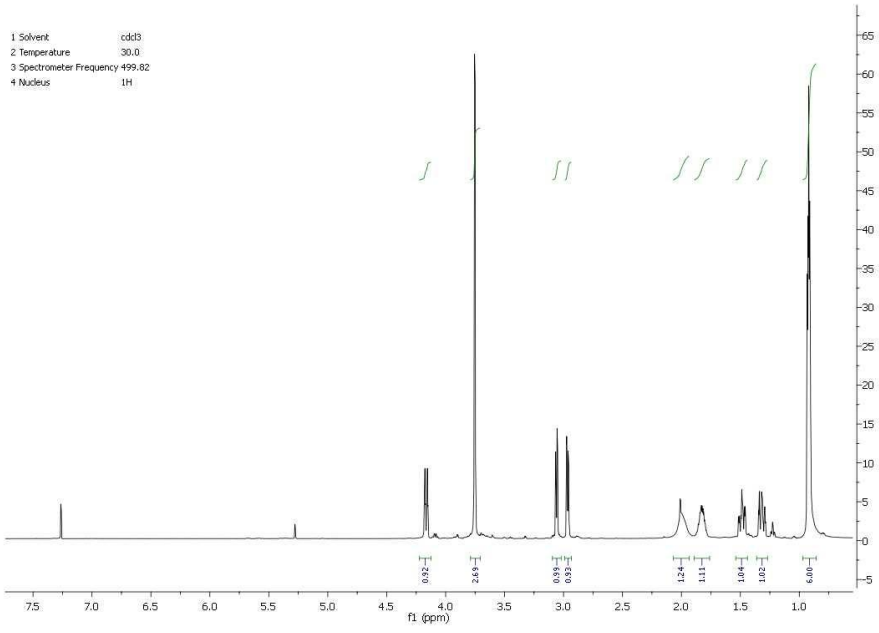

170.04
77.33
77.07
76.82
68.99
59.38
52.48
52.47
49.29
35.12
18.85
13.74
f1 (ppm)

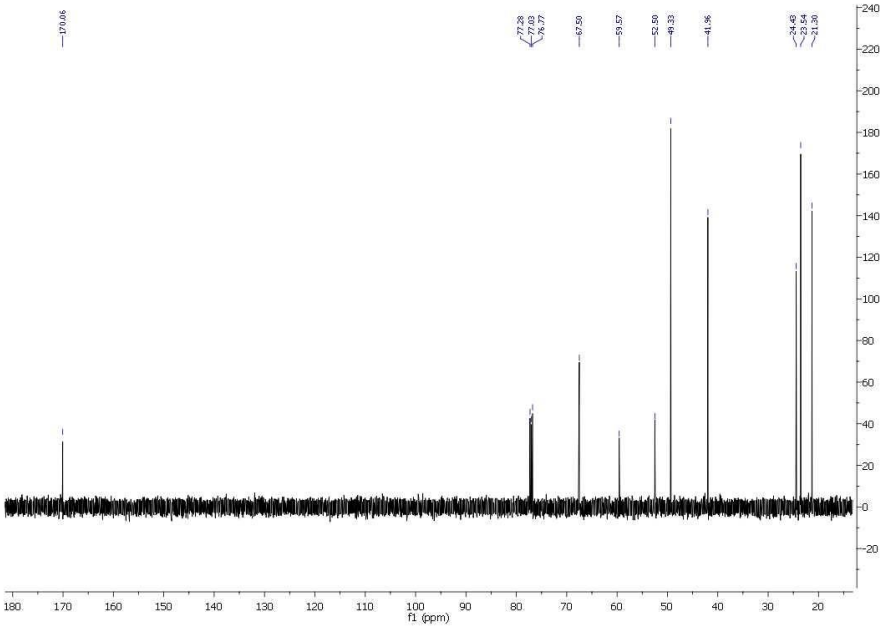

OH
O
OMe
O
3.2d

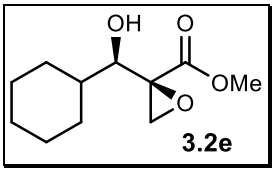

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
0.99
2.66
0.95
0.91
1.30
2.09
6.00
f1 (ppm)

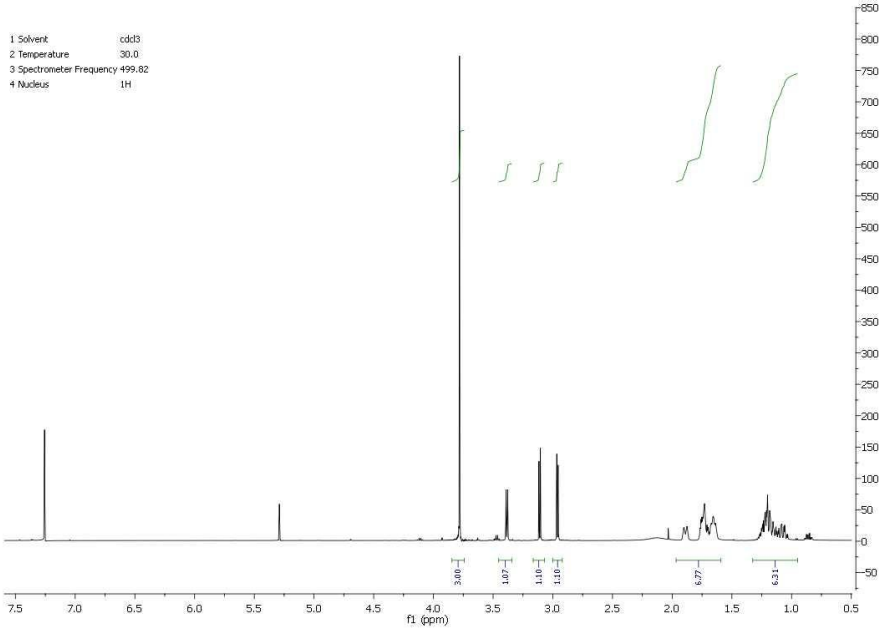

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
170.01
77.32
77.07
76.81
67.92
59.46
52.46
52.44
41.64
41.81
24.41
21.51
21.39
f1 (ppm)

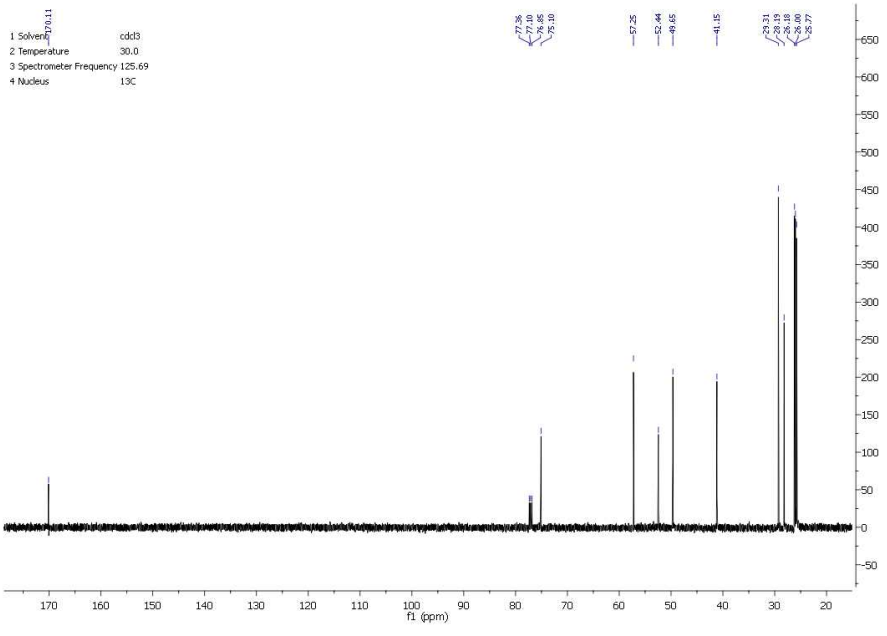

OH
O
OMe
O
3.3d

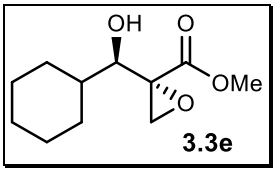

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
0.92
2.69
0.99
0.93
1.24
1.11
1.04
1.02
6.00
f1 (ppm)

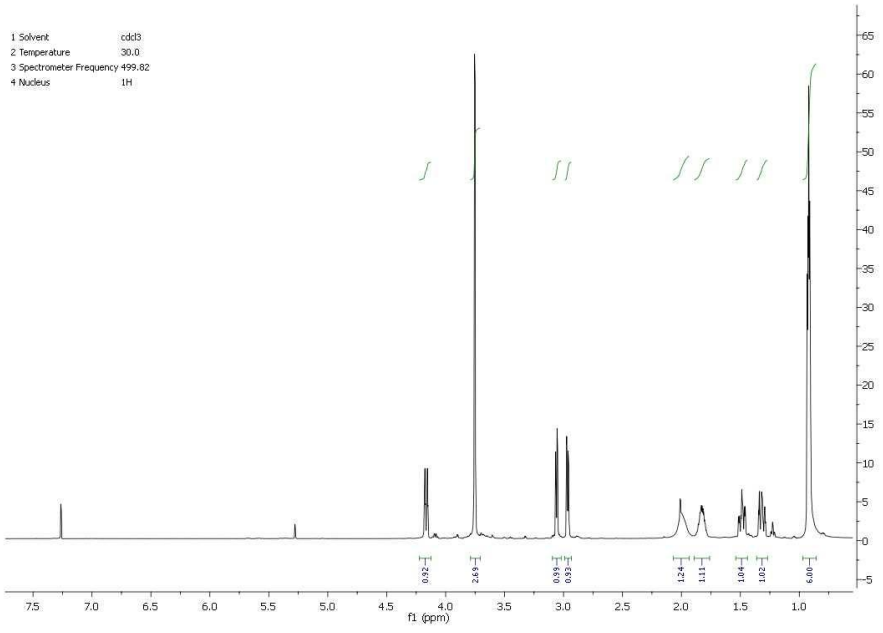

170.06
77.28
77.00
76.77
67.50
59.57
52.50
49.33
41.96
24.48
23.54
21.30
f1 (ppm)

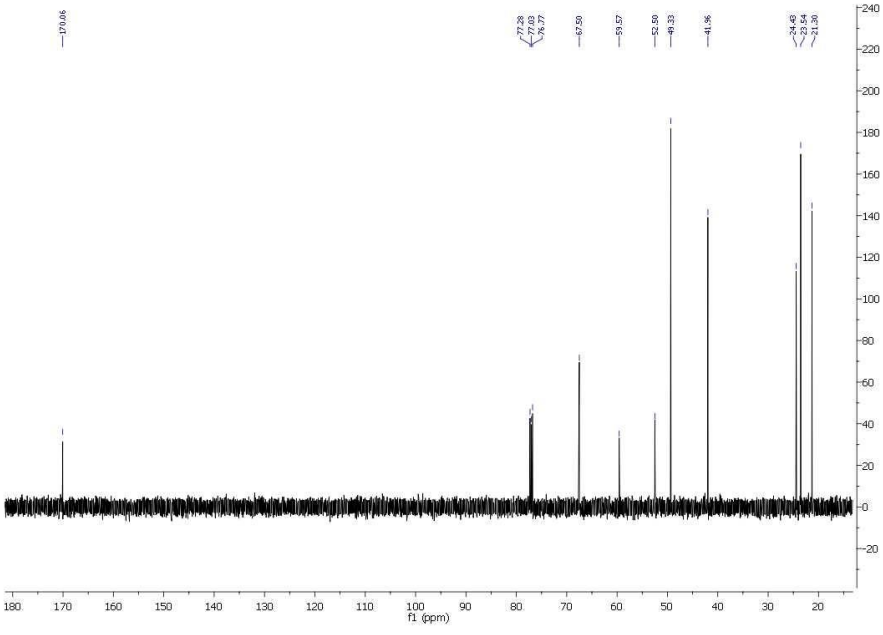

OH
O
OMe
O
3.2e

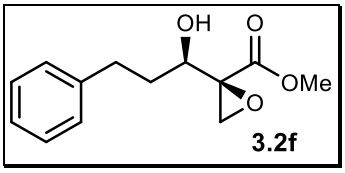

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
3.00
1.07
1.10
1.10
6.77
6.31
f1 (ppm)

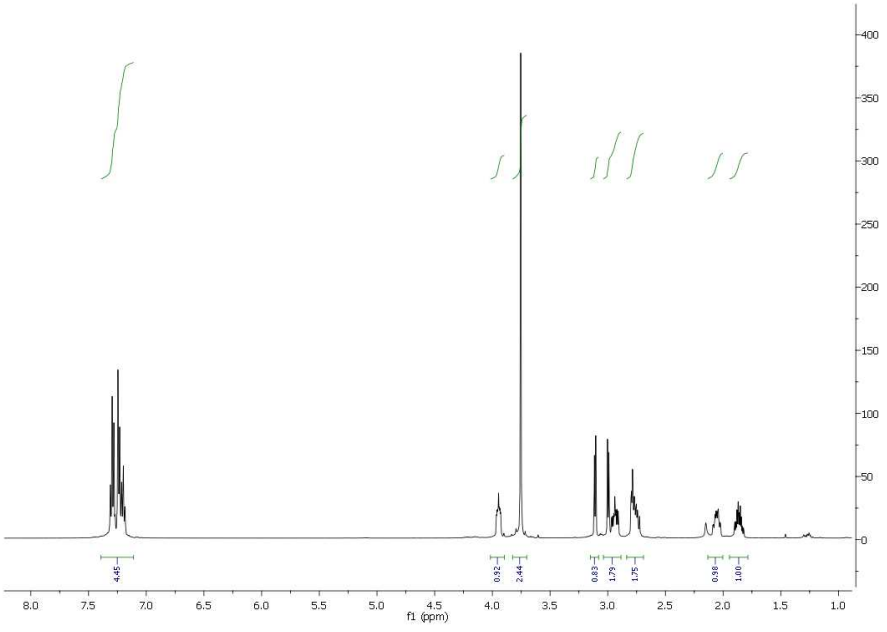

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
170.11
77.36
77.10
76.85
75.10
57.25
52.44
49.65
41.15
29.31
28.19
26.18
26.00
25.77
f1 (ppm)

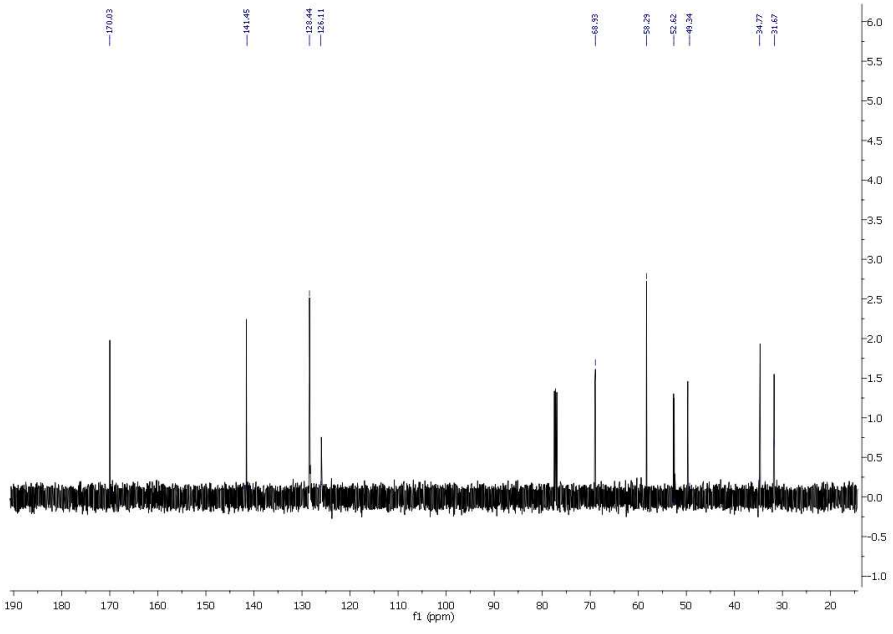

OH
O
OMe
O
3.3e

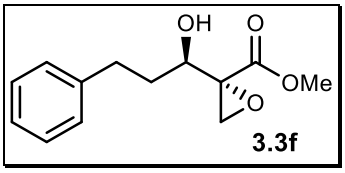

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

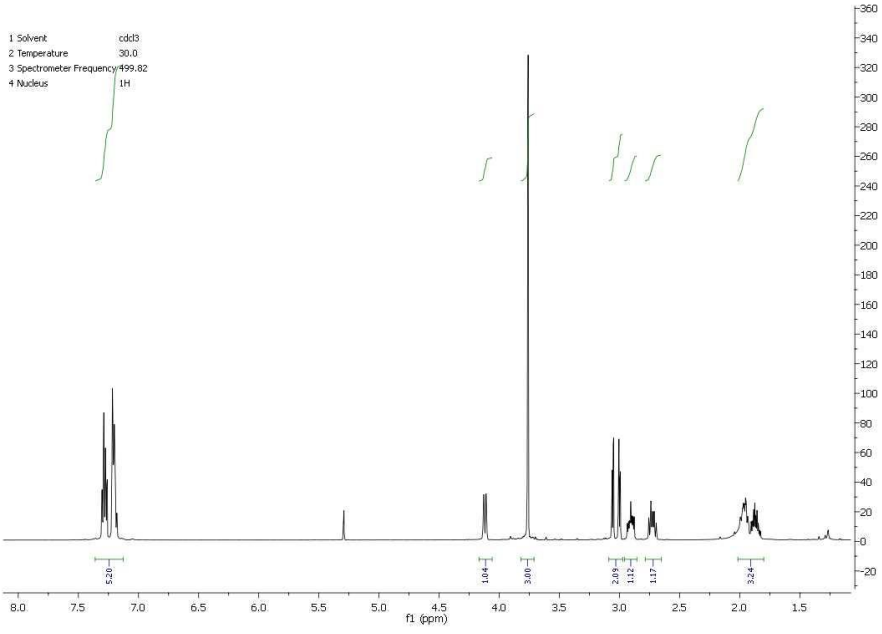

170.06
77.28
77.00
76.77
67.50
59.57
52.50
49.33
41.96
24.43
23.54
21.30
f1 (ppm)

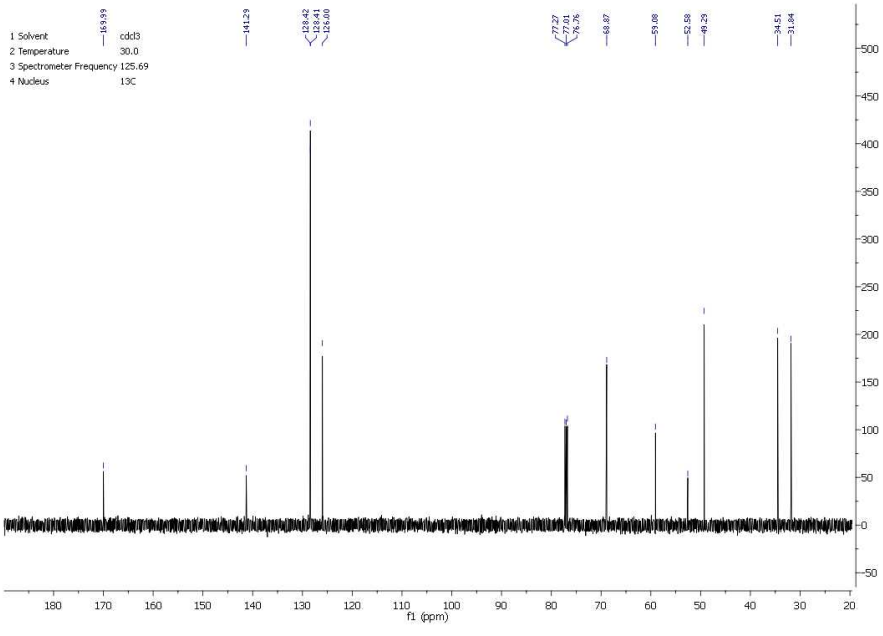

OH
O
OMe
O
3.2f

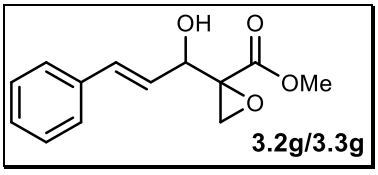

4.45
0.92
2.44
0.83
1.79
1.75
0.98
1.00
f1 (ppm)

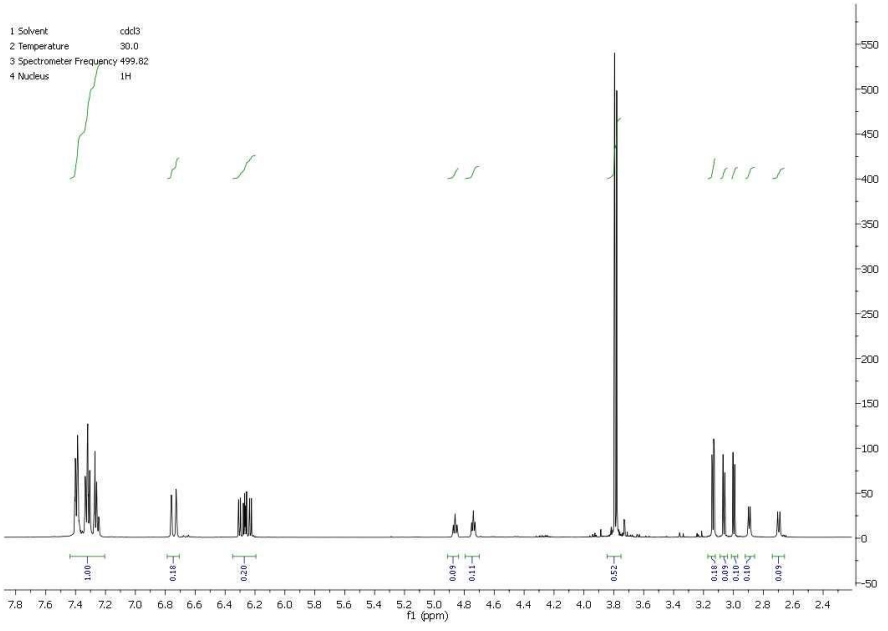

170.03
141.45
128.44
126.11
68.93
58.29
52.62
49.34
34.77
31.67
f1 (ppm)

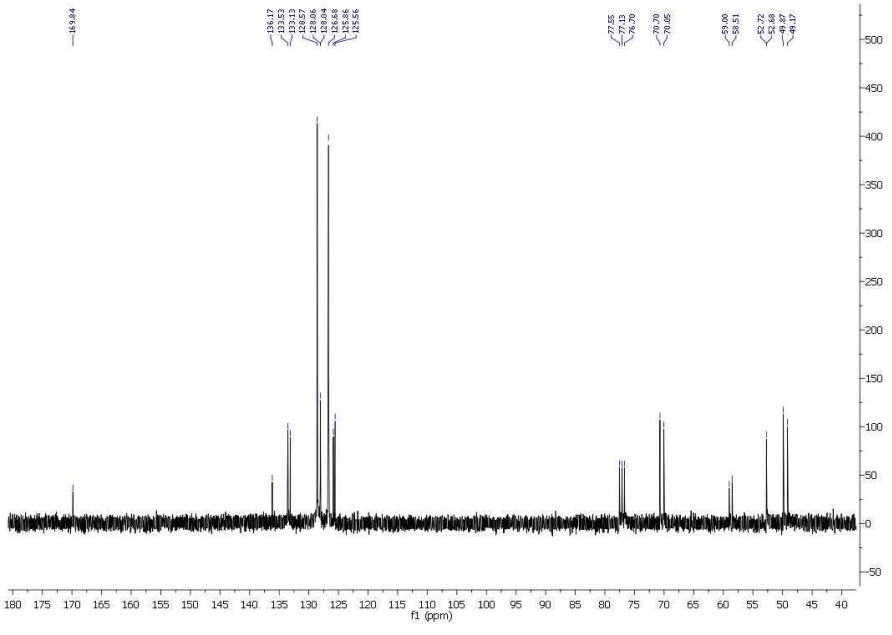

OH
O
OMe
O
3.3f

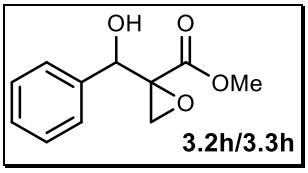

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
5.20
1.04
3.00
2.09
1.12
1.17
3.24
f1 (ppm)

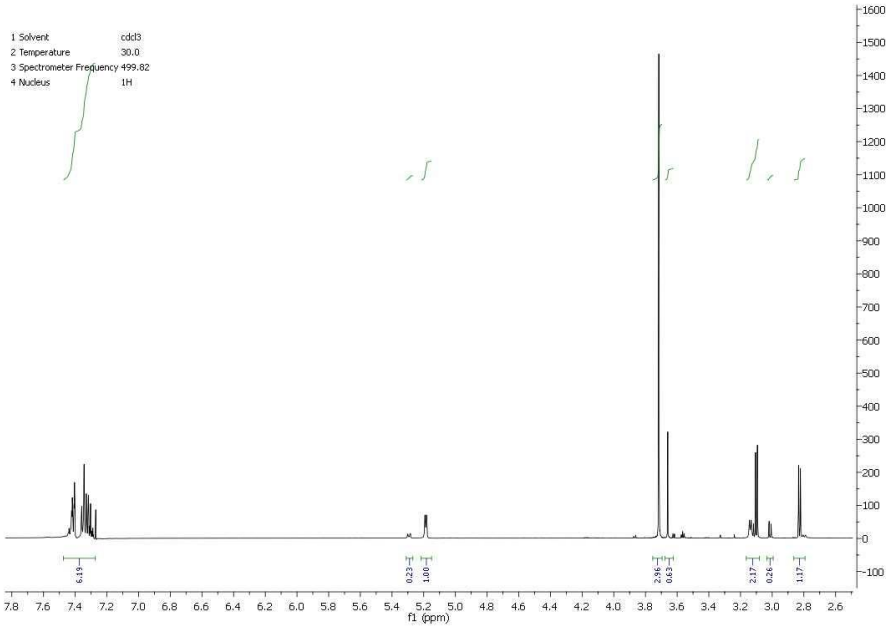

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
169.99
141.29
128.42
128.41
126.00
77.27
77.01
76.76
68.87
59.08
52.58
49.23
34.51
31.84
f1 (ppm)

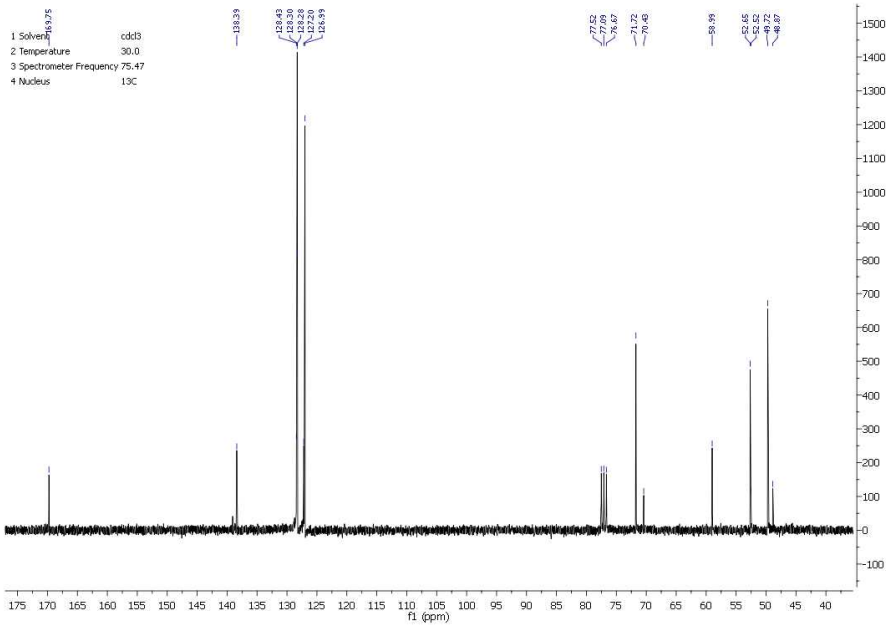

OH
O
OMe
O
3.2g/3.3g

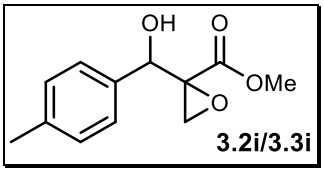

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

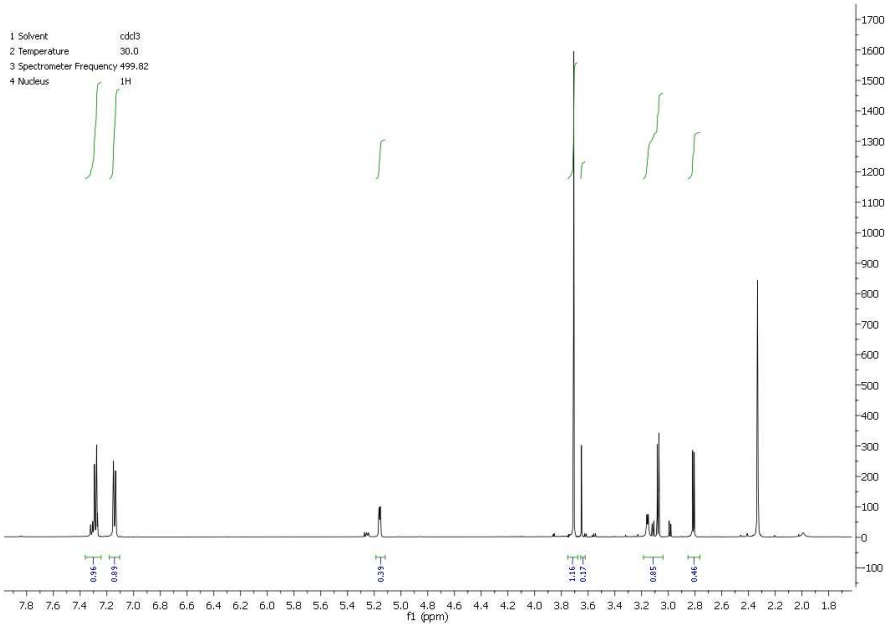

f1 (ppm)

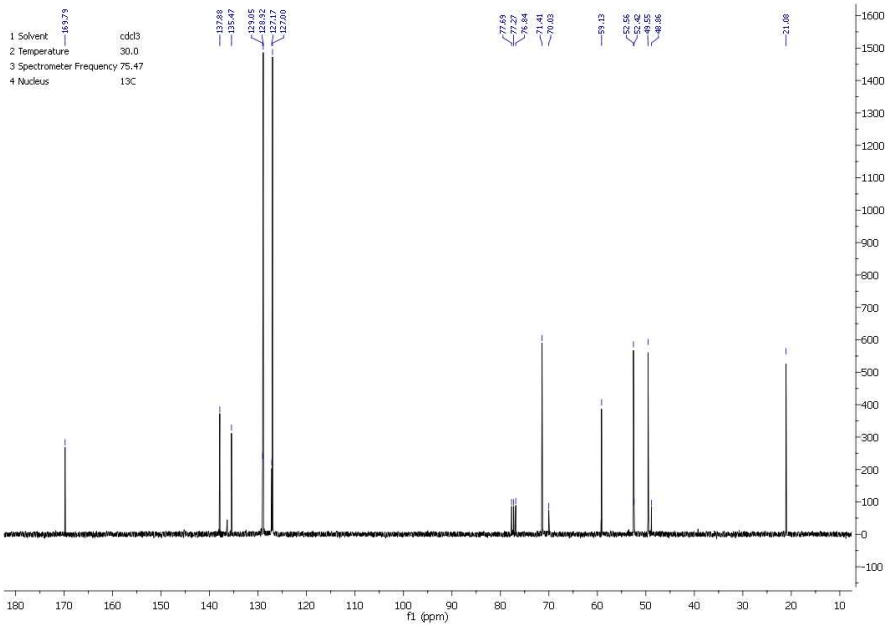

OH
O
OMe
O
3.2h/3.3h

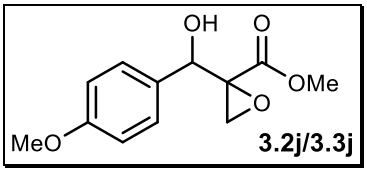

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
6.19
0.23
1.00
2.86
0.63
2.17
0.26
1.17
f1 (ppm)

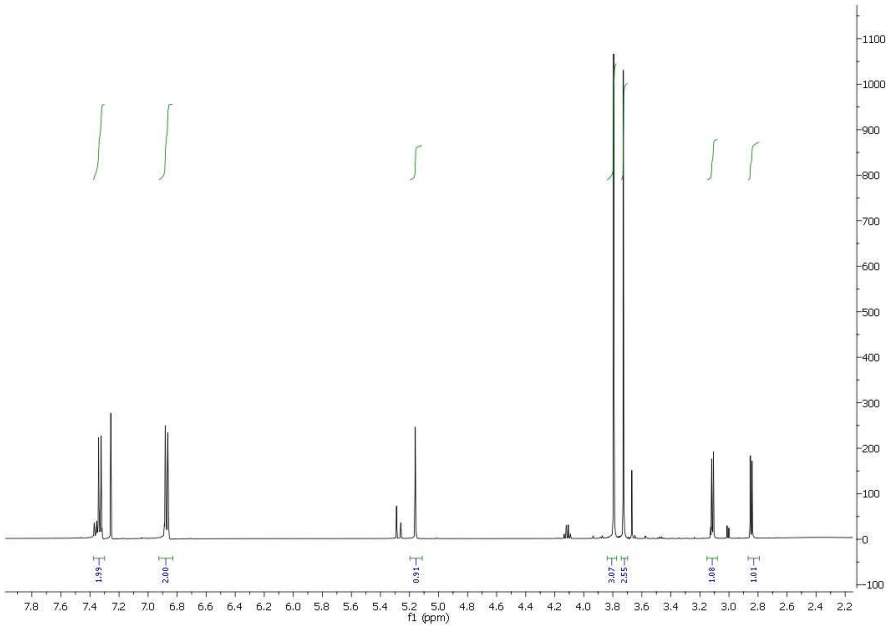

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 75.47
4 Nucleus 13C
169.75
138.39
128.43
128.30
128.28
127.20
126.99
77.52
77.09
76.67
71.72
70.48
58.99
52.65
52.32
48.72
48.67
f1 (ppm)

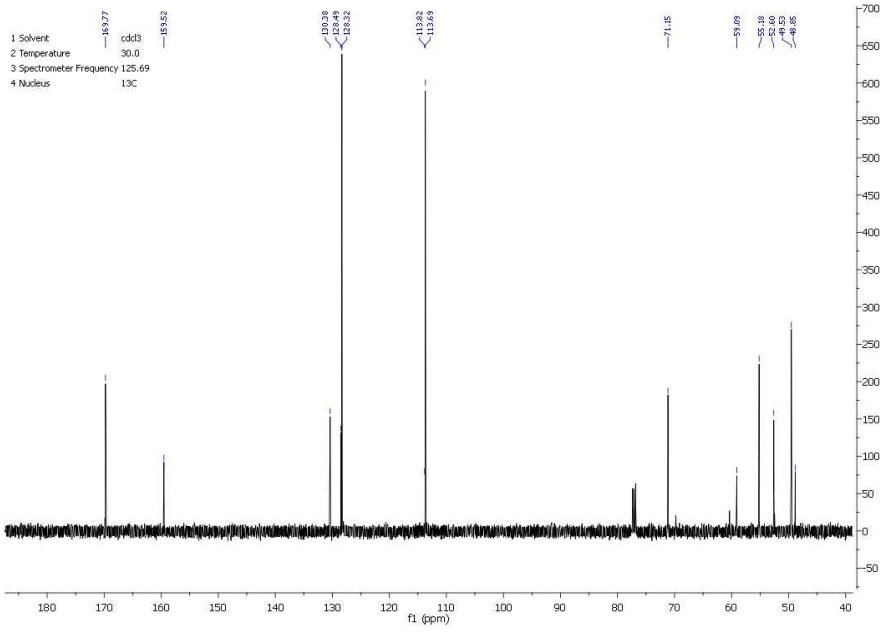

OH
O
OMe
O
3.2i/3.3i

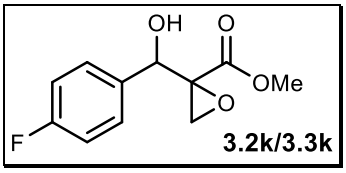

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

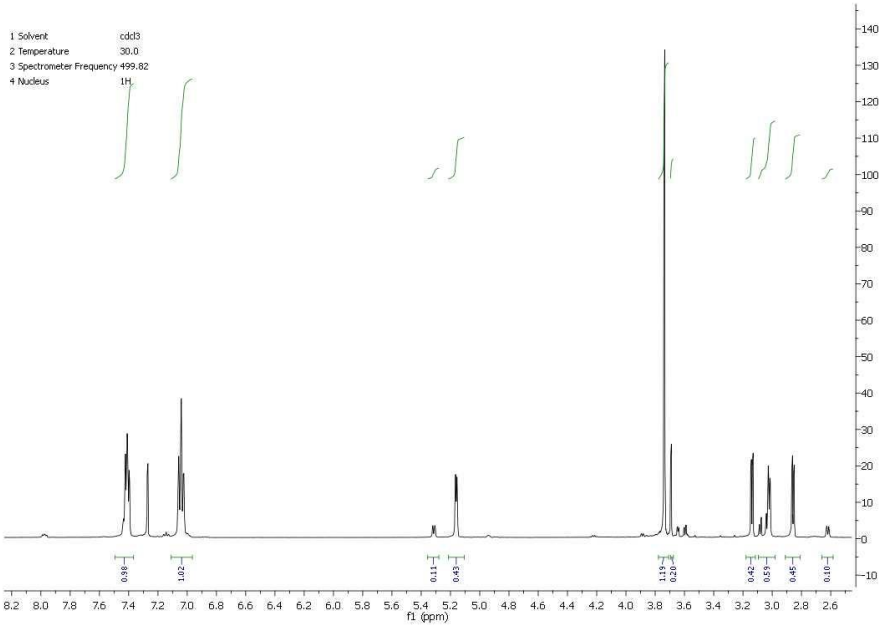

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 75.47
4 Nucleus 13C
f1 (ppm)

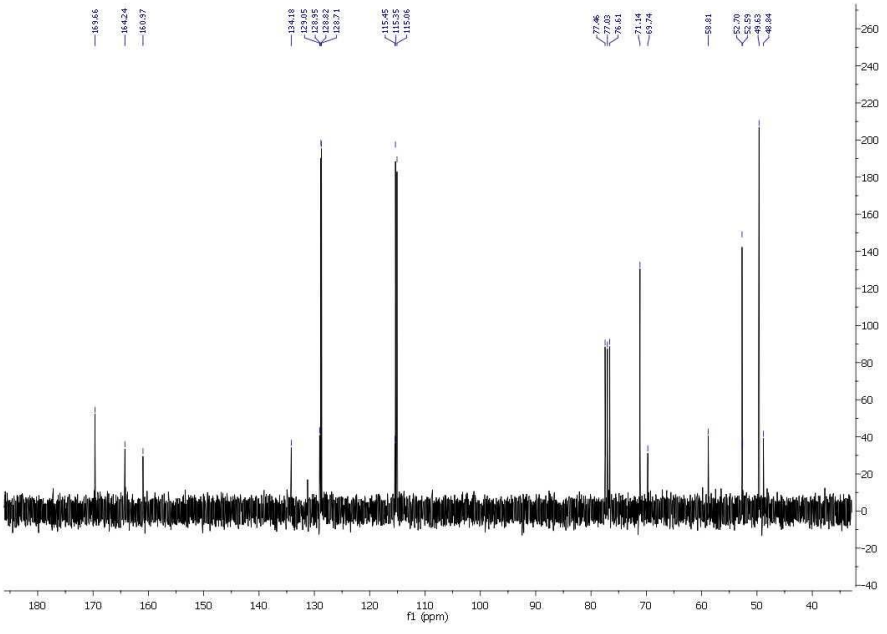

OH
O
OMe
O
MeO
3.2j/3.3j

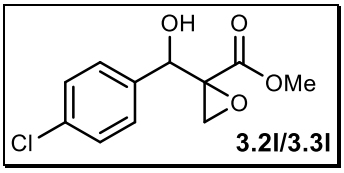

1.59
2.00
0.93
3.07
2.55
1.08
1.01
f1 (ppm)

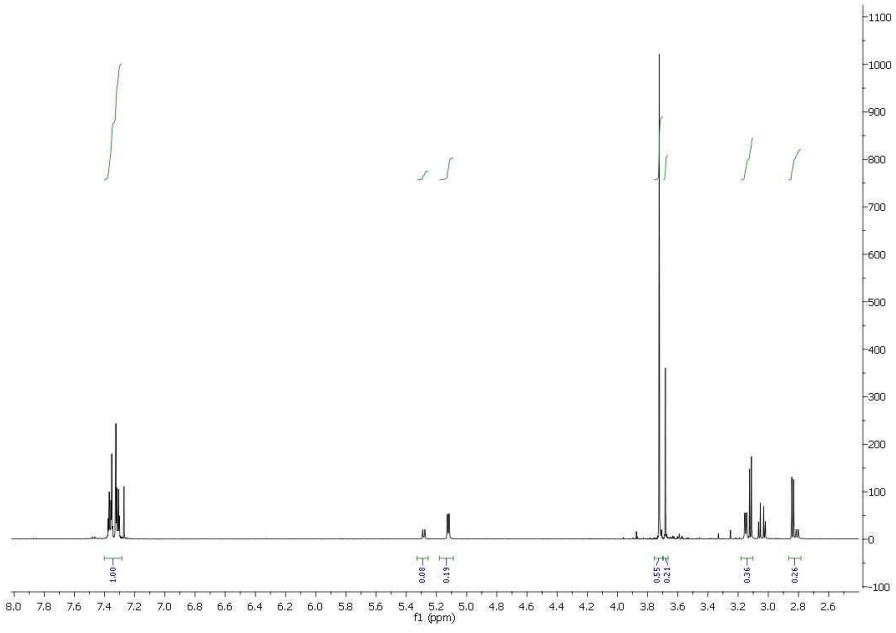

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
169.77
159.52
130.38
129.49
128.32
113.82
113.69
71.15
59.09
55.18
52.60
48.53
40.85
f1 (ppm)

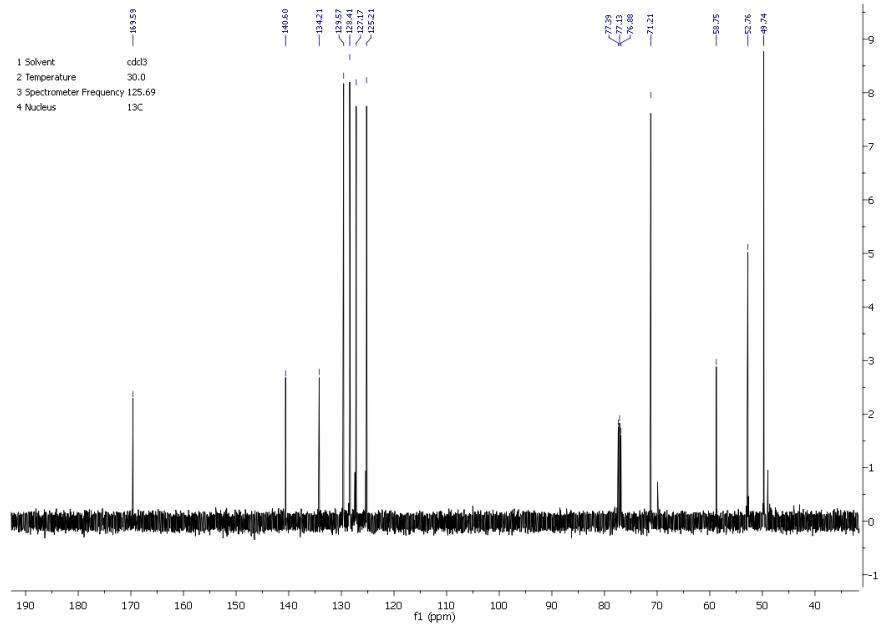

OH
O
OMe
O
F
3.2k/3.3k

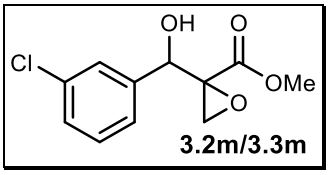

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

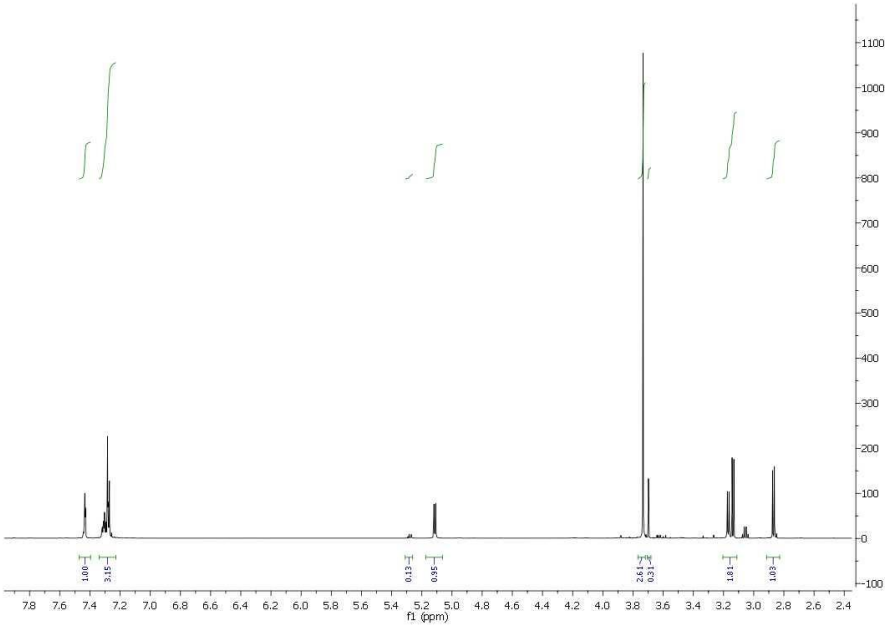

163.68
164.24
160.97
134.18
129.95
128.82
128.71
115.45
115.35
115.06
77.46
77.03
76.61
71.14
69.74
59.81
52.70
52.59
49.63
48.84
f1 (ppm)

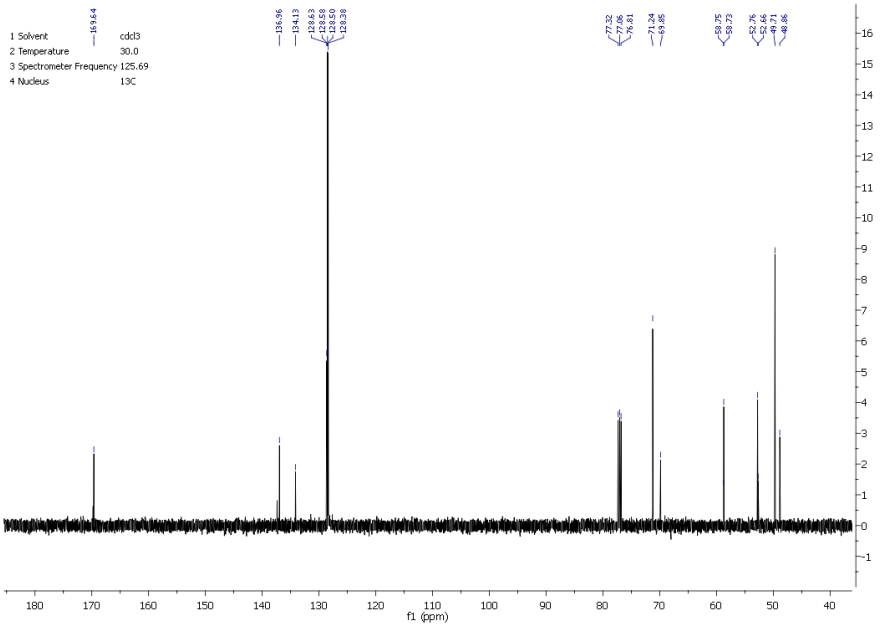

OH
O
OMe
O
Cl
3.2l/3.3l

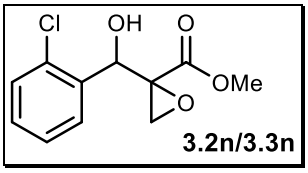

1.00
0.08
0.19
0.55
0.21
0.36
0.26
f1 (ppm)

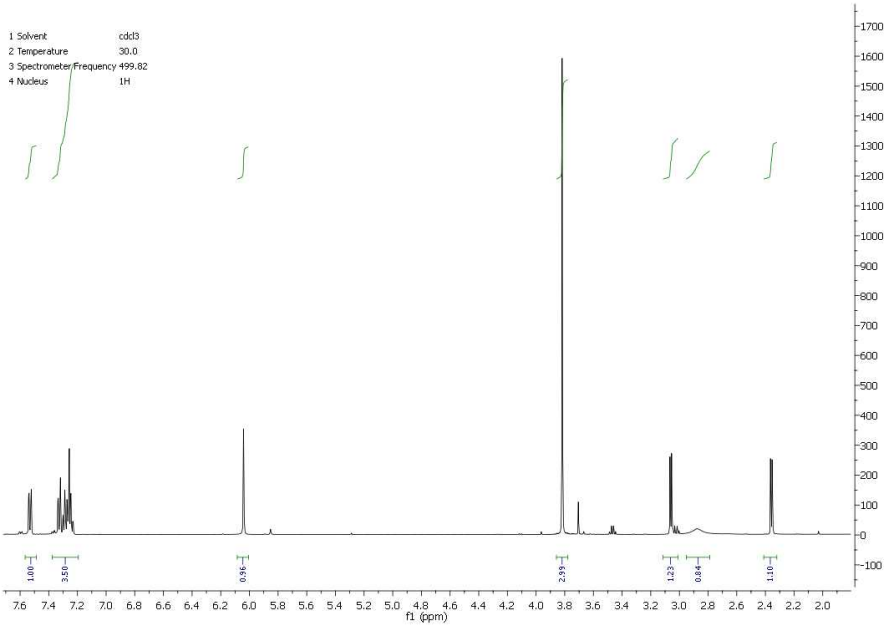

163.59
140.60
134.21
129.57
128.41
127.17
125.21
77.39
77.13
76.88
71.21
59.75
52.76
49.74
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

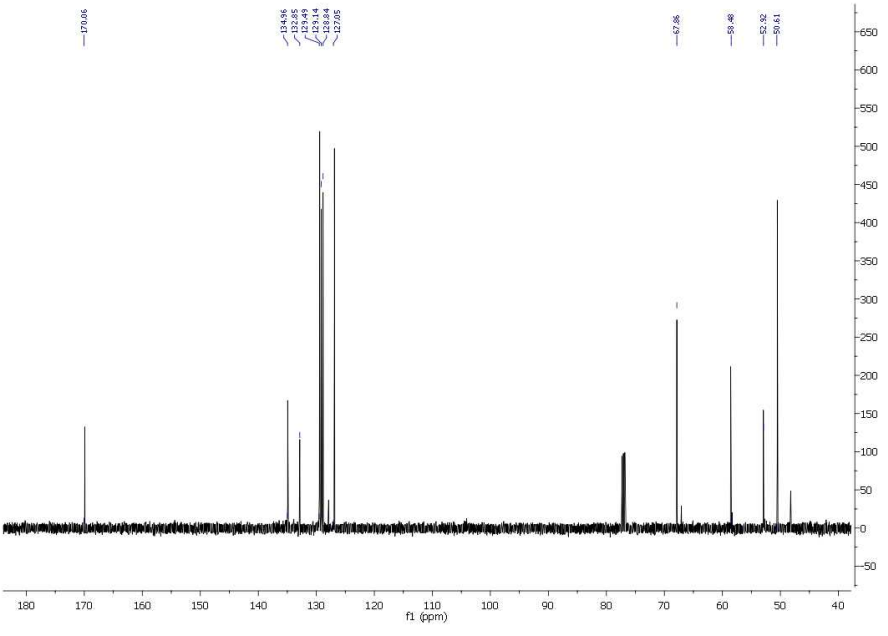

OH
O
Cl
OMe
O
3.2m/3.3m

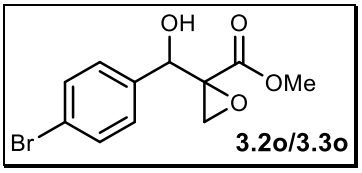

1.00
3.15
0.13
0.95
2.61
0.91
1.81
1.03
f1 (ppm)


169.64
136.96
134.13
128.63
128.58
128.50
126.58
77.32
77.06
76.81
71.24
69.85
59.75
59.73
52.76
52.46
49.71
48.86
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

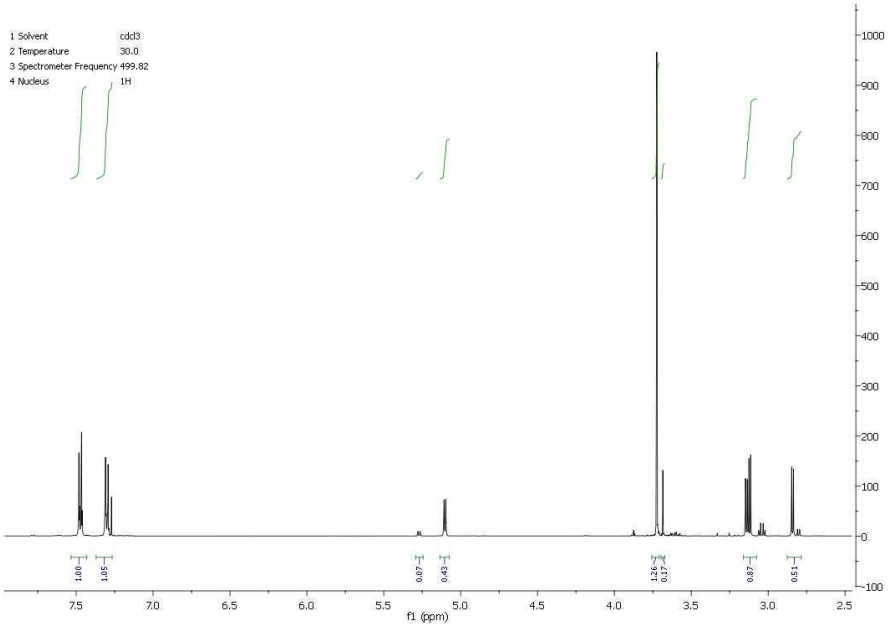

Cl
OH
O
OMe
O
3.2n/3.3n

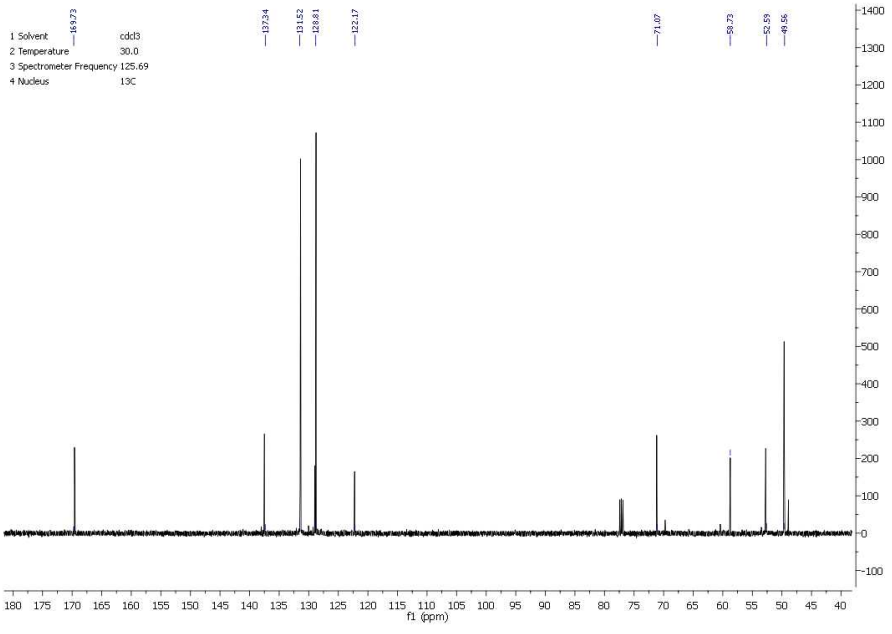

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer/Frequency 499.82
4 Nucleus 1H
1.00
3.50
0.96
2.99
1.23
0.84
1.10
f1 (ppm)

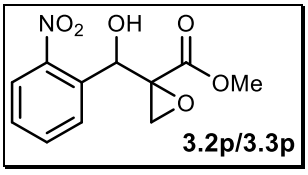

170.06
134.96
132.85
129.48
129.14
128.84
127.05
67.86
58.48
52.92
59.61
f1 (ppm)

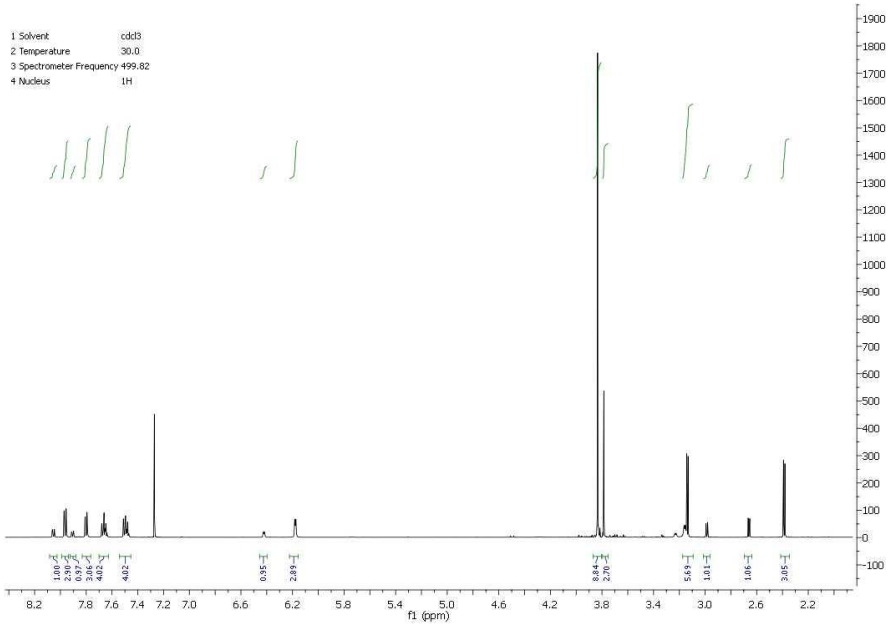

OH
O
OMe
O
Br

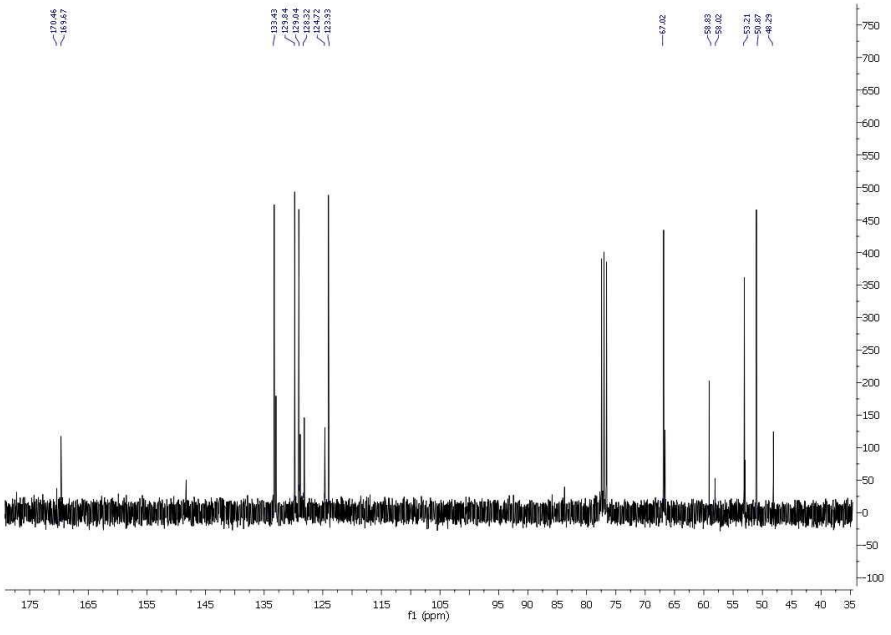
**3.2o/3.3o**

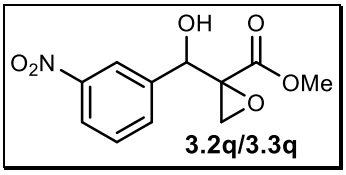

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
1.00
1.05
0.07
0.43
1.26
0.17
0.87
0.61
f1 (ppm)

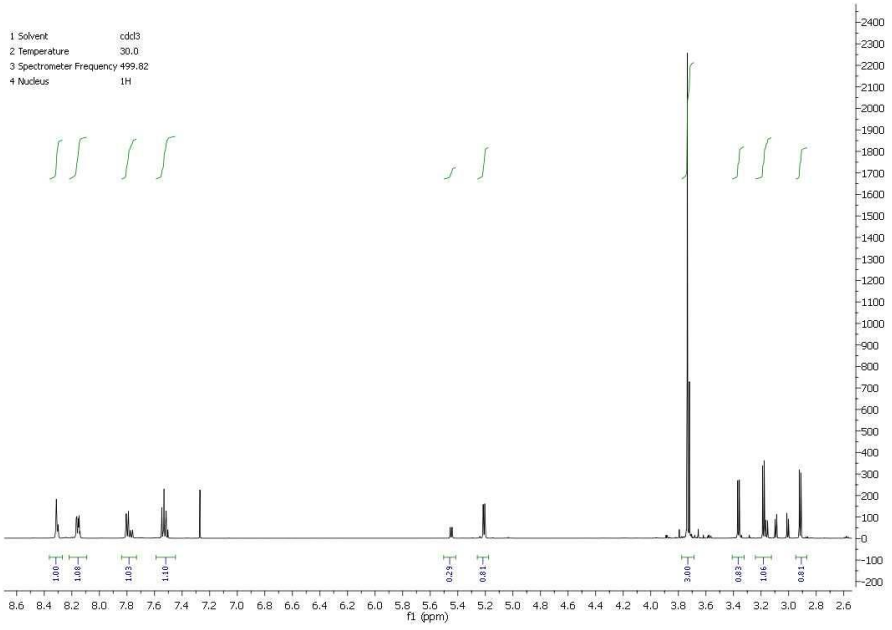

169.73
137.34
131.52
128.81
122.17
71.07
59.73
52.59
49.56
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

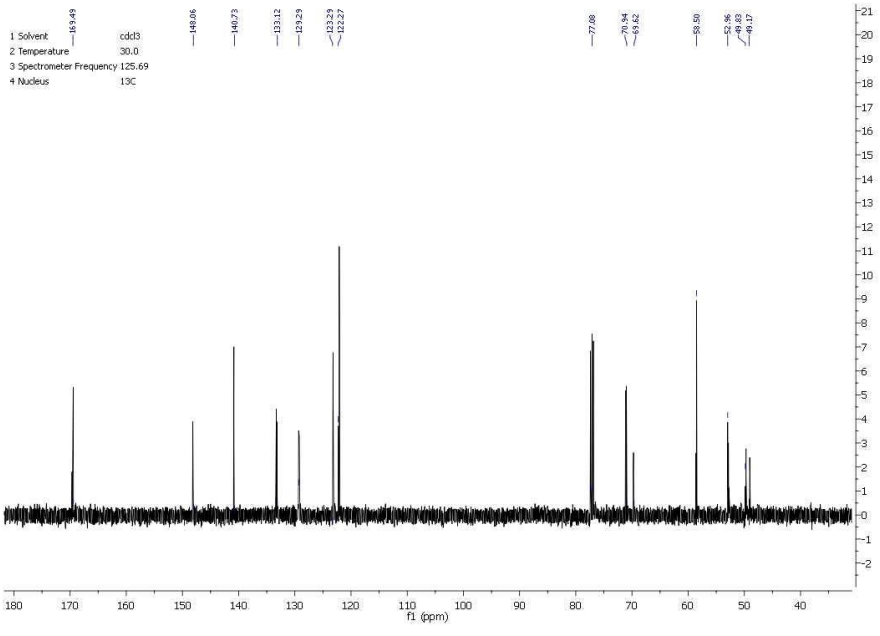

NO2
OH
O
OMe
O
3.2p/3.3p

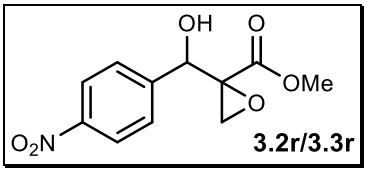

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

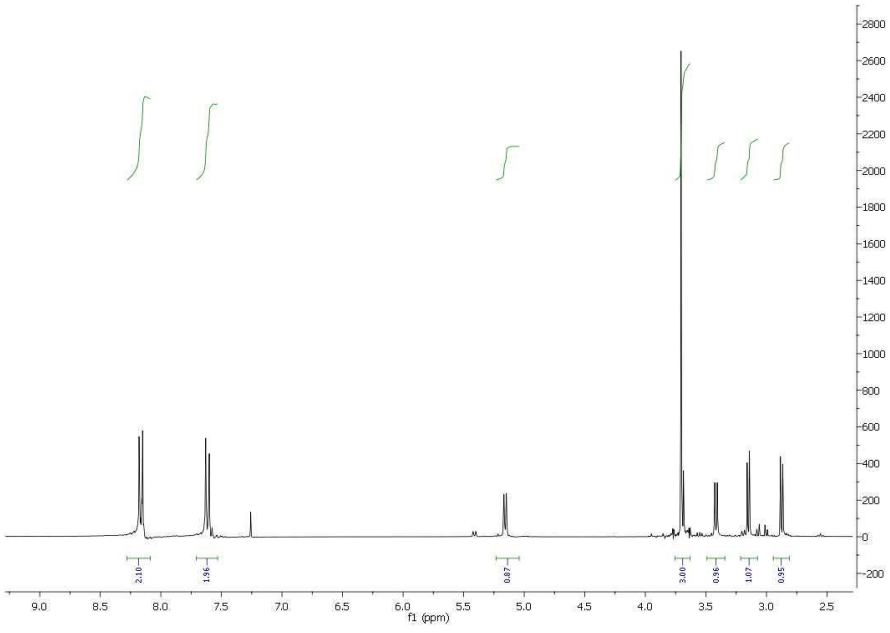

170.46
165.67
133.43
129.84
129.04
128.32
124.72
123.93
67.02
58.83
58.02
53.21
50.87
48.29
f1 (ppm)

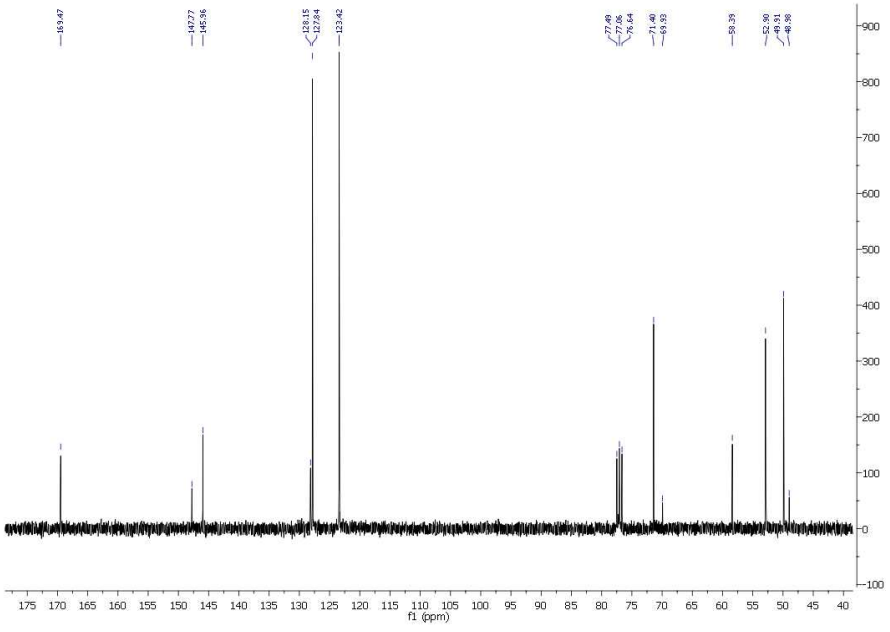

OH
O
$O_2N$
OMe
O
3.2q/3.3q

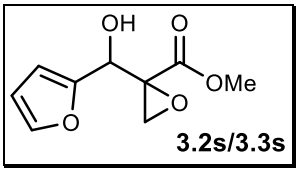

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

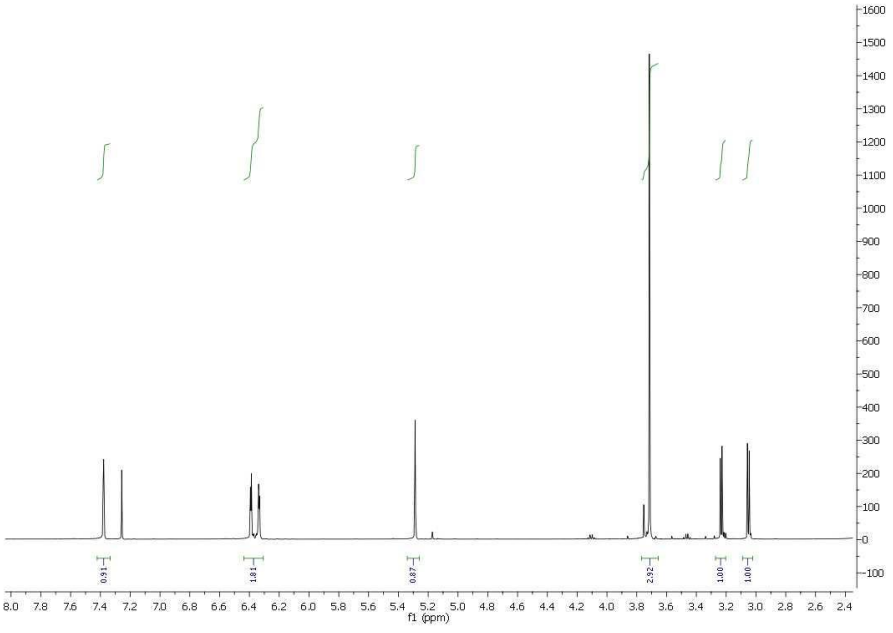

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
163.49
148.06
140.73
133.12
123.29
123.29
122.27
77.00
70.94
69.62
58.50
52.96
49.03
46.17
f1 (ppm)

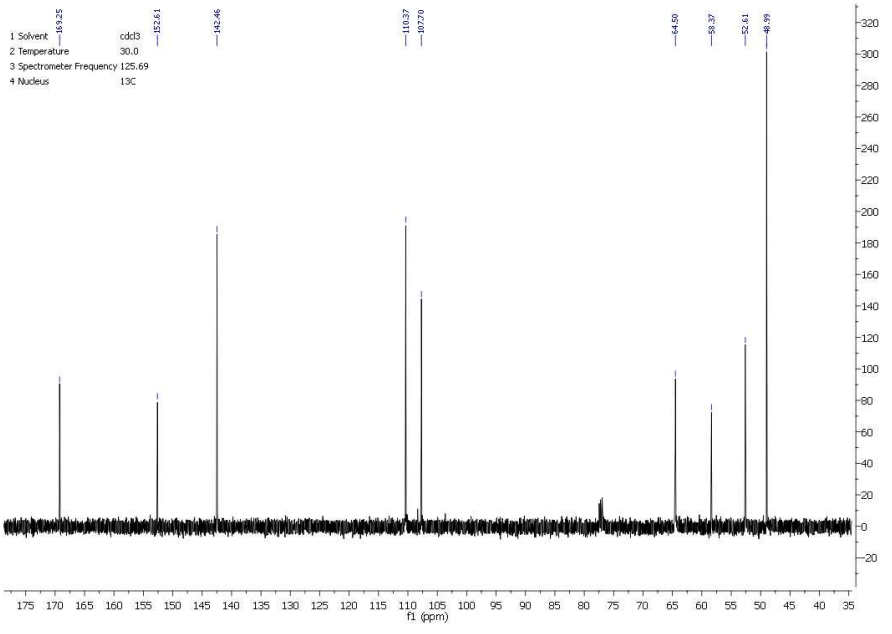

OH
O
OMe
O
$O_2N$
3.2r/3.3r

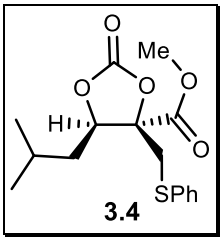

2.10
1.96
0.87
3.00
0.96
1.07
0.95
f1 (ppm)


169.47
147.27
145.96
128.15
127.84
123.42
77.49
77.06
76.64
71.40
69.93
59.39
52.90
49.91
48.98
f1 (ppm)

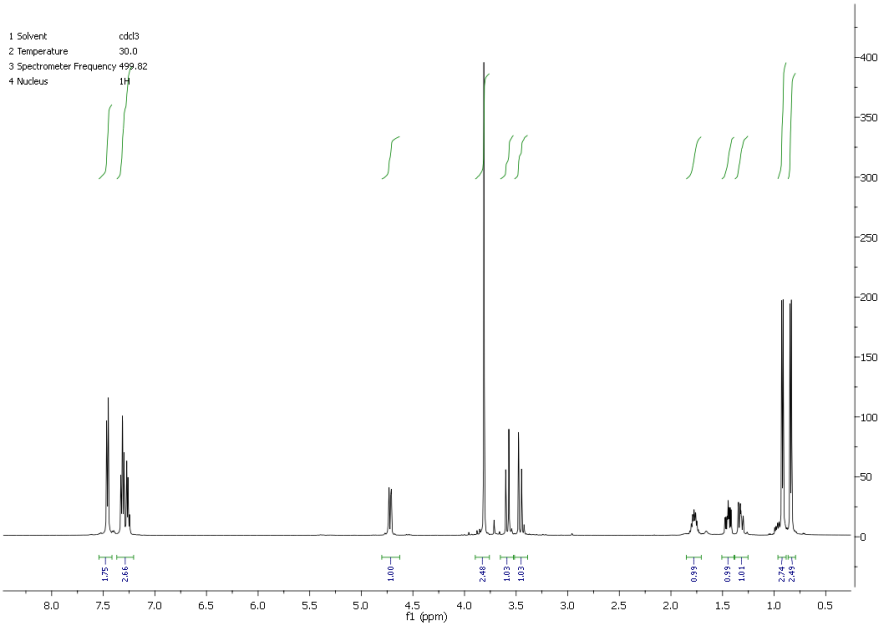

OH
O
OMe
O
O
3.2s/3.3s

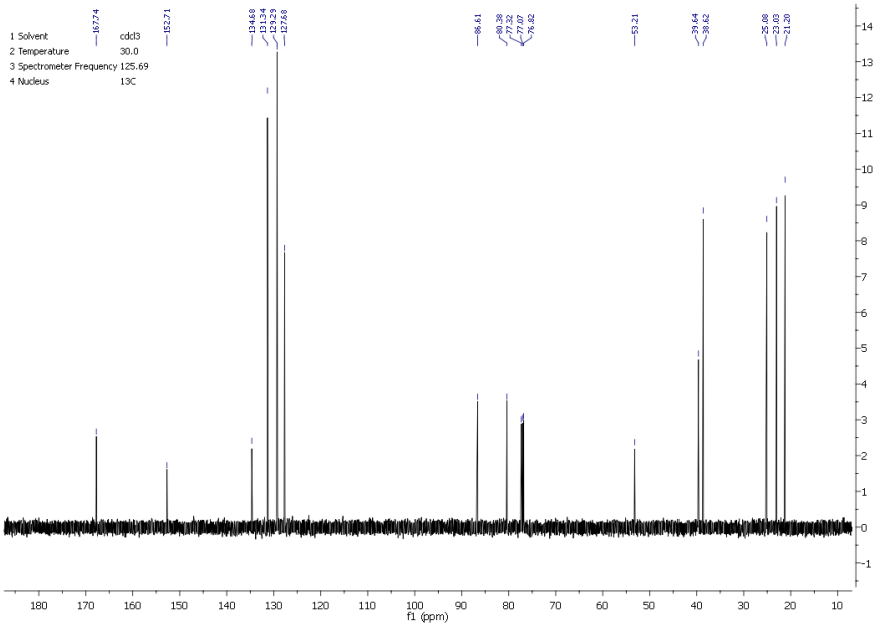

0.91
1.81
0.87
2.92
1.00
1.00
f1 (ppm)

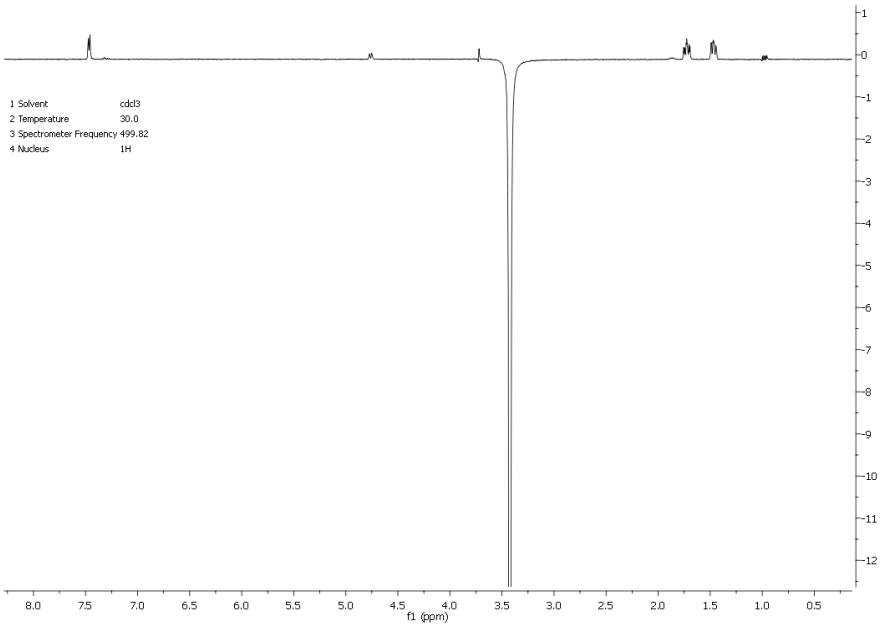

169.25
152.61
142.46
110.37
107.70
64.50
58.37
52.61
48.99
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

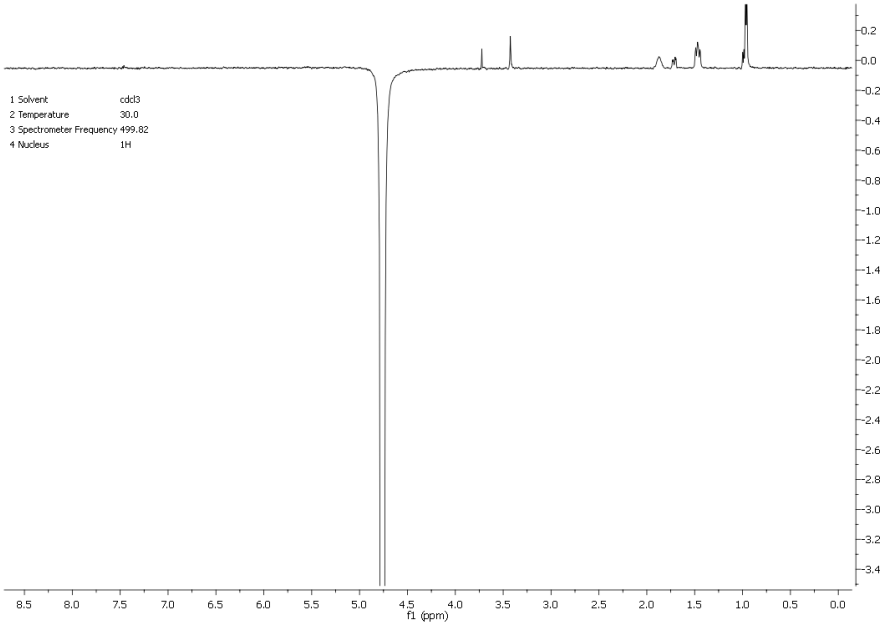

O
Me
O
O
O
H
O
SPh

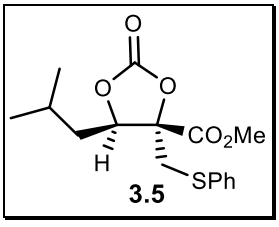
**3.4**

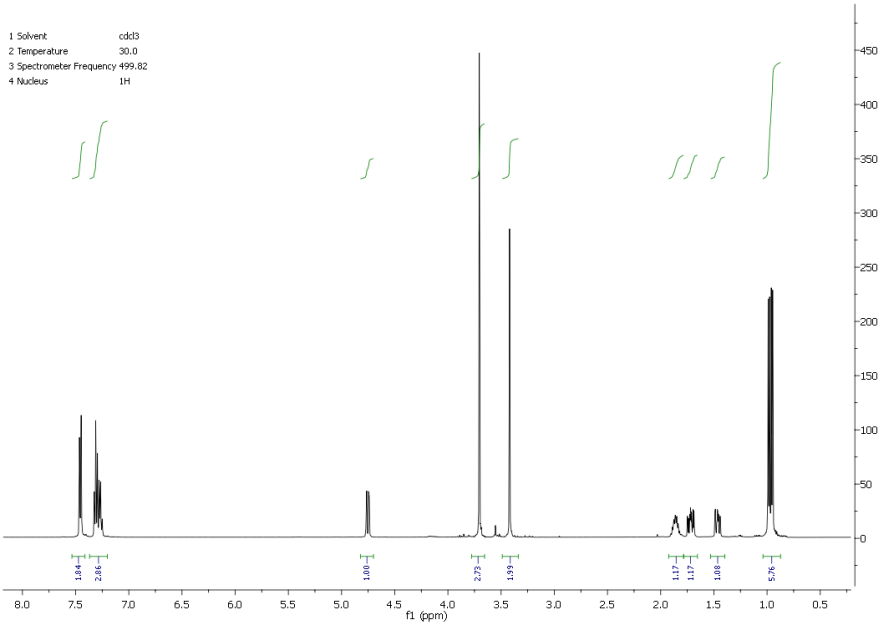

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
1.75
2.66
1.00
2.48
1.03
1.03
0.99
0.99
1.01
2.74
2.49
f1 (ppm)

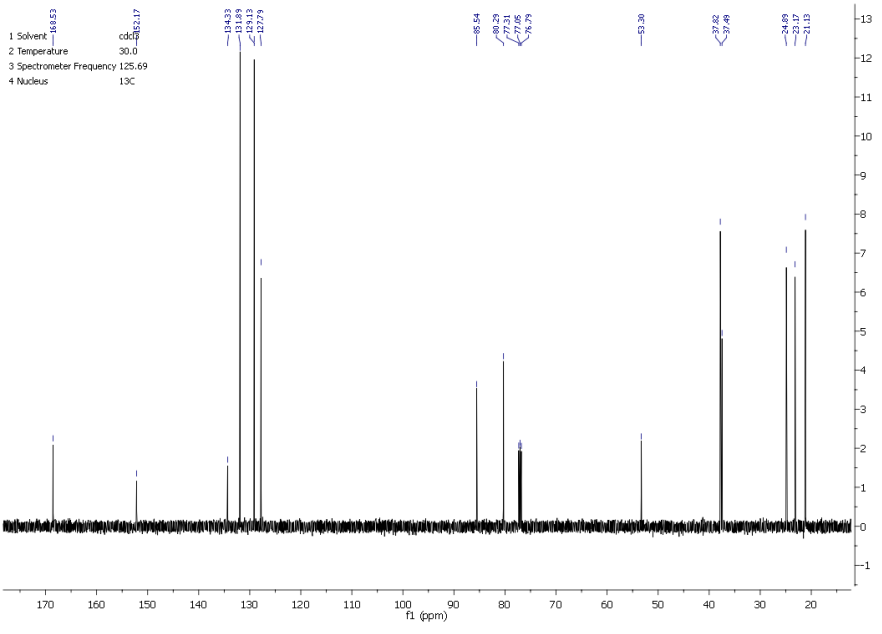

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
167.74
152.71
134.68
131.34
129.29
127.68
86.61
80.38
77.32
77.07
76.82
53.21
39.64
38.62
25.08
23.03
21.26
f1 (ppm)

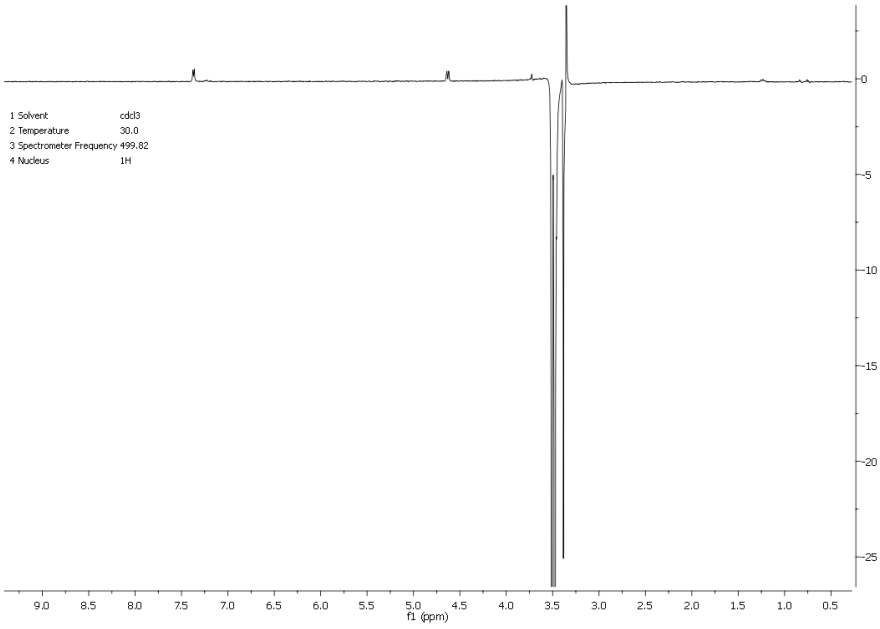

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

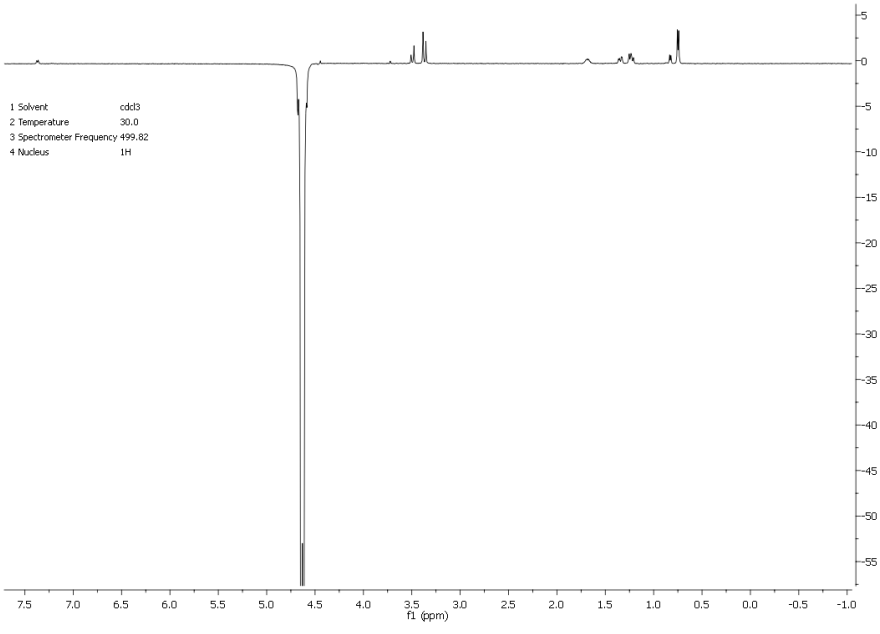

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

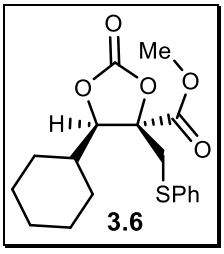

O
O
O
CO2Me
H
SPh
3.5


1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

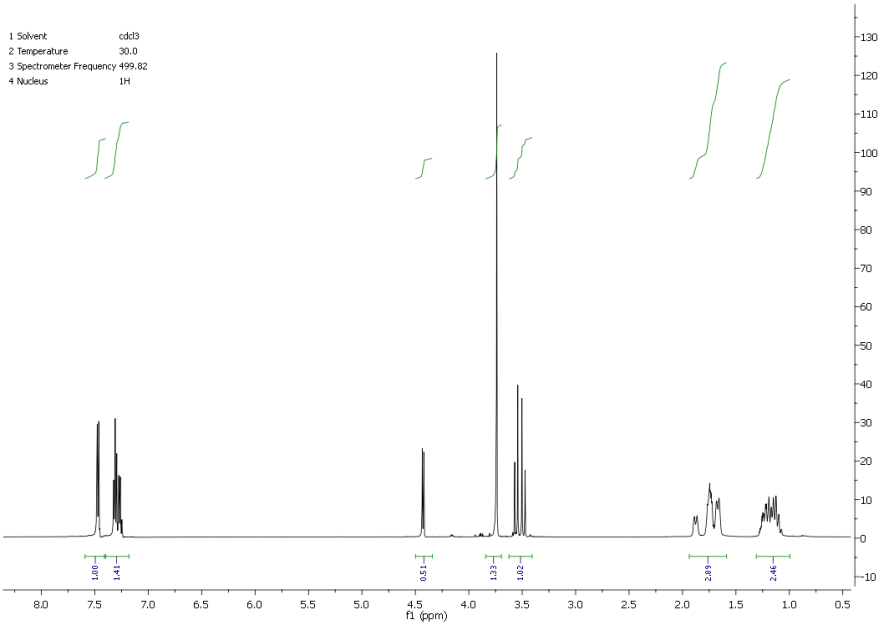

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

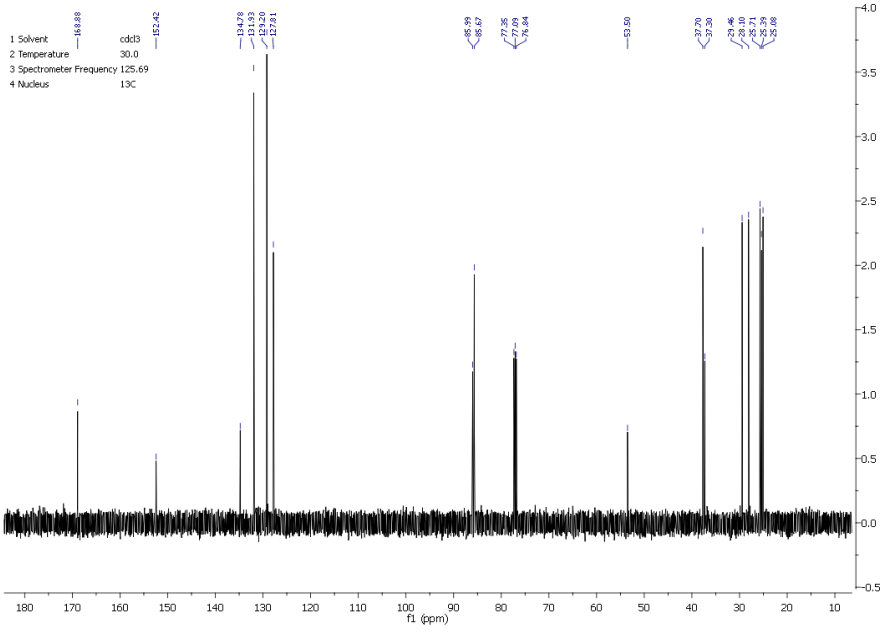

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

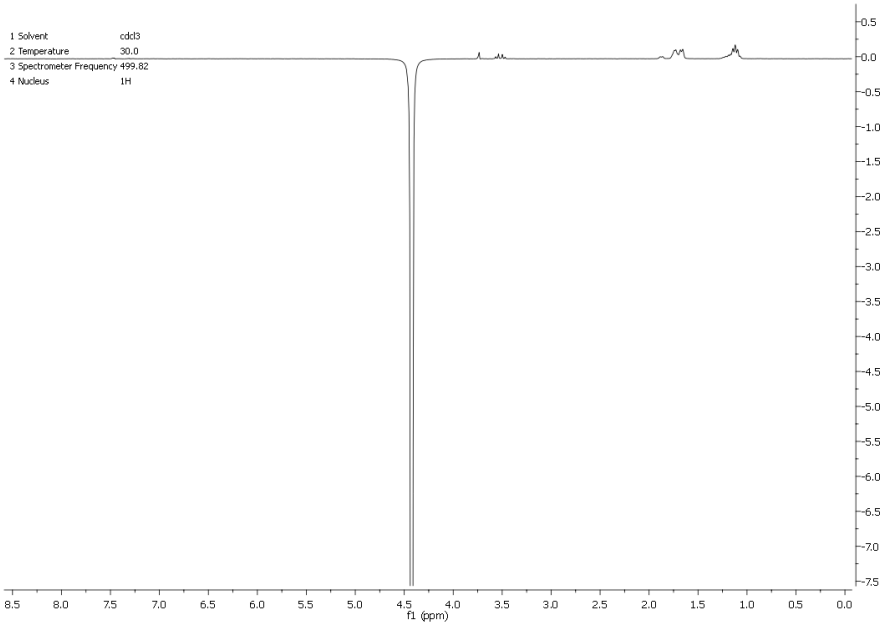

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

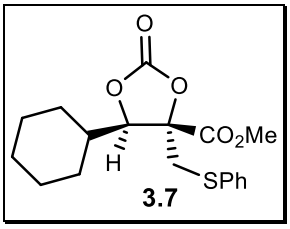

O
Me
O
O
O
H
O
SPh

**3.6**

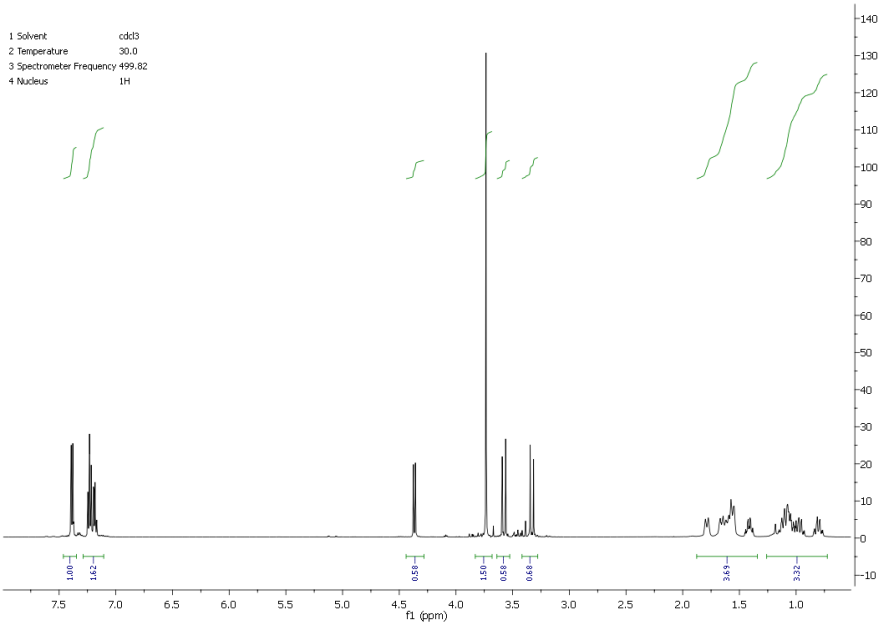

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
1.00
1.41
0.51
1.33
1.02
2.89
2.46
f1 (ppm)

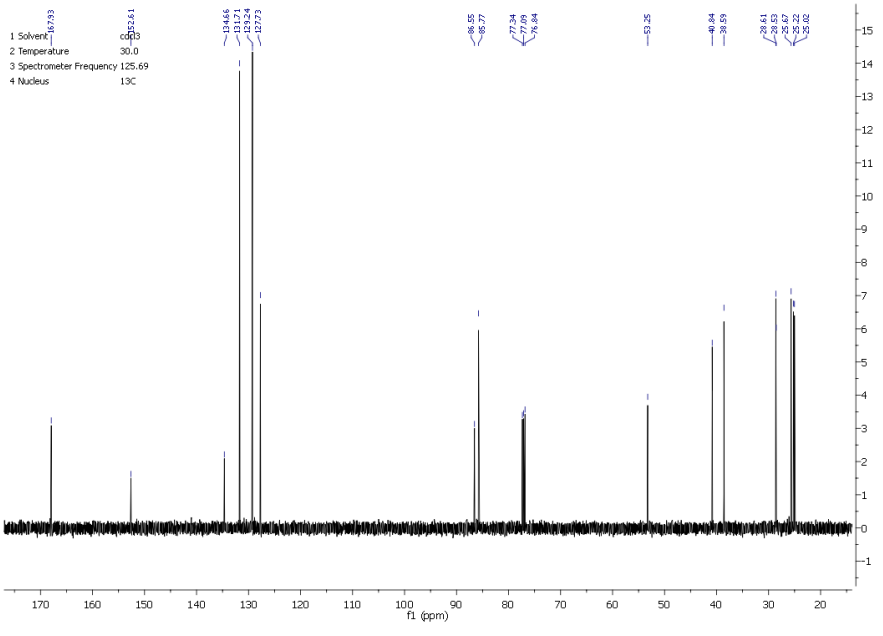

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
169.88
152.42
134.78
131.53
129.20
127.81
85.99
85.67
77.25
77.00
76.84
53.50
27.70
27.30
f1 (ppm)

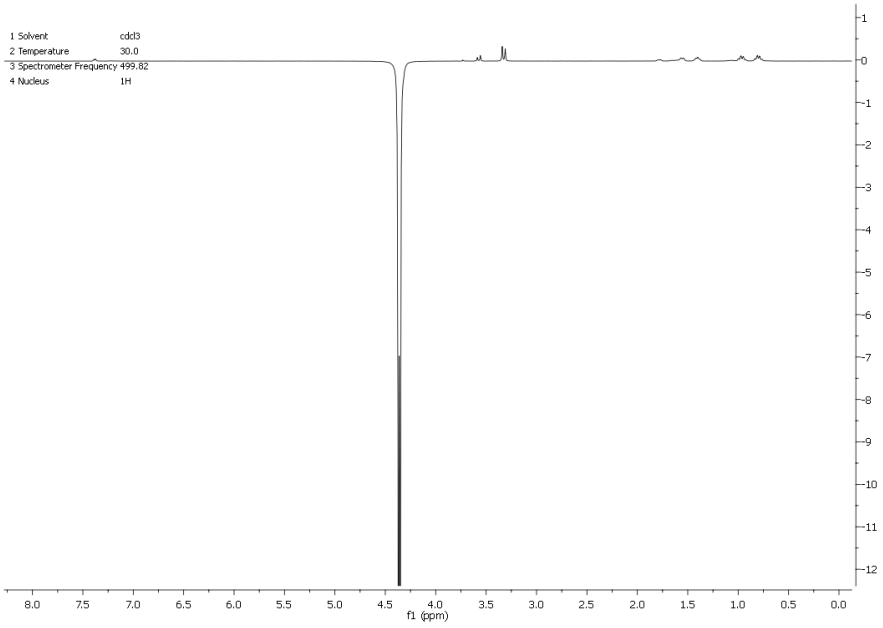

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)


O
O
O
CO2Me
H
SPh

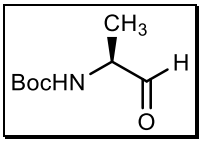
**3.7**

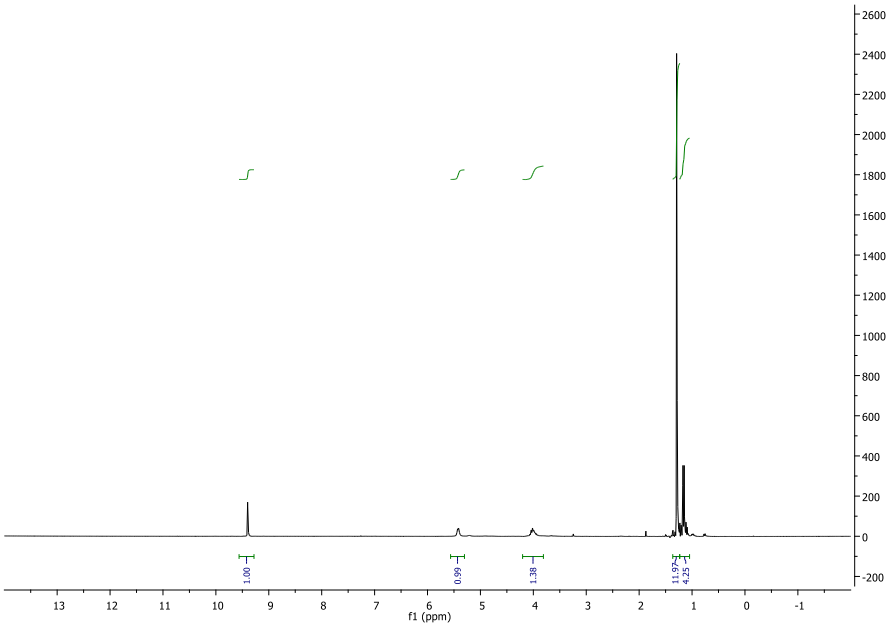

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
1.00
1.62
0.58
1.50
0.58
0.68
3.69
3.32
f1 (ppm)

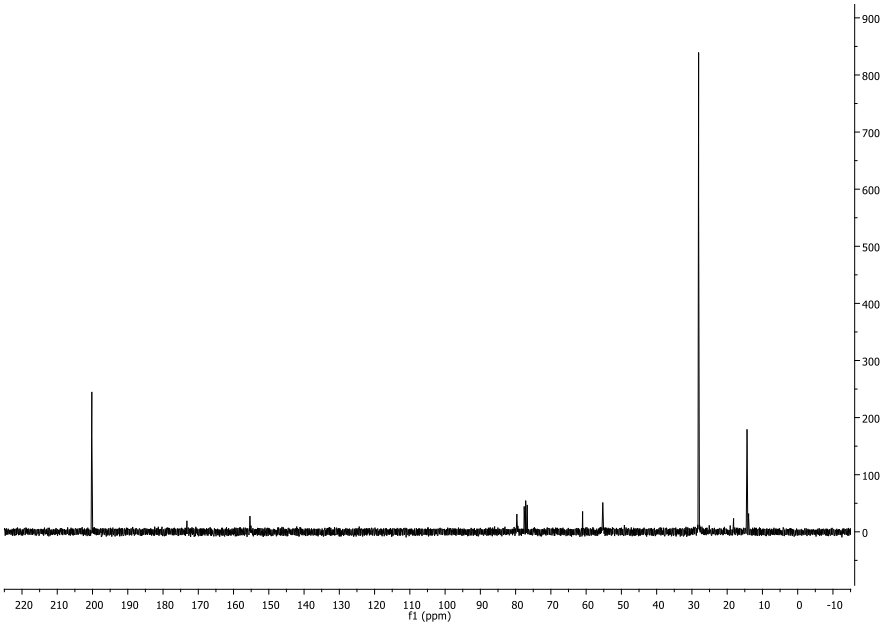

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
167.93
152.61
134.66
131.71
129.24
127.73
86.55
85.77
77.34
77.09
76.84
53.25
40.84
38.59
29.61
28.53
25.47
25.22
25.02
f1 (ppm)

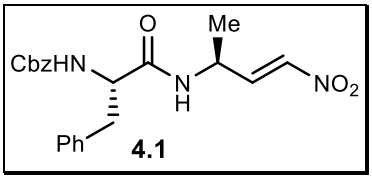

1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)
1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12

# Spectra Chapter 4:

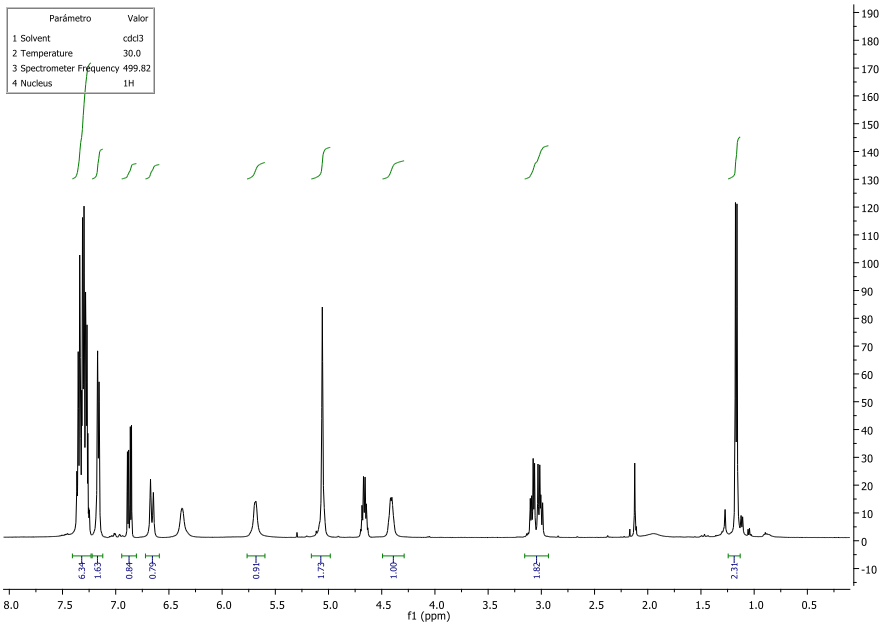

CH3
BocHN
H
O

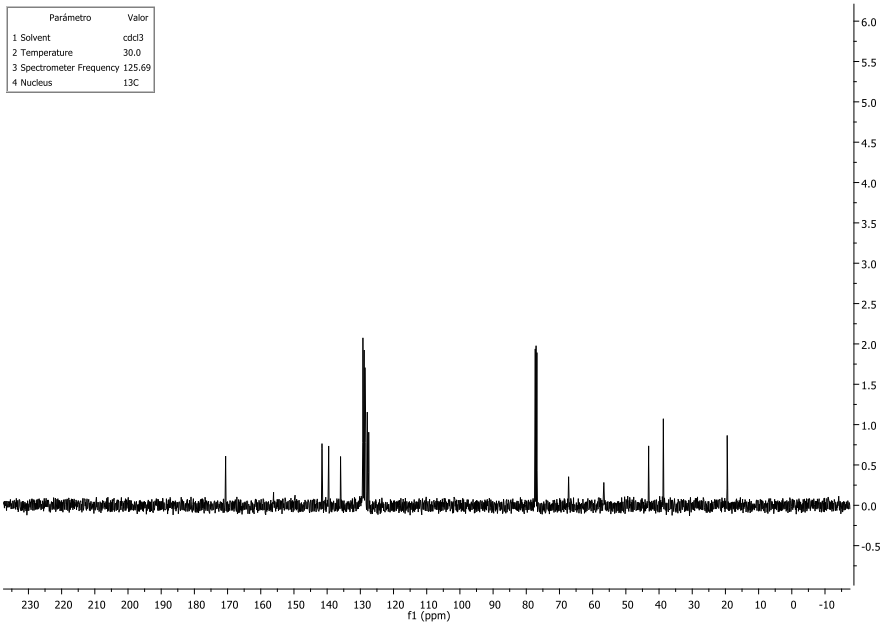

1.00
0.99
1.38
11.97
4.25
f1 (ppm)

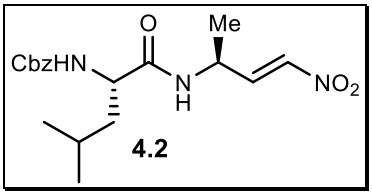

f1 (ppm)


O
Me
CbzHN
N
H
NO2
Ph

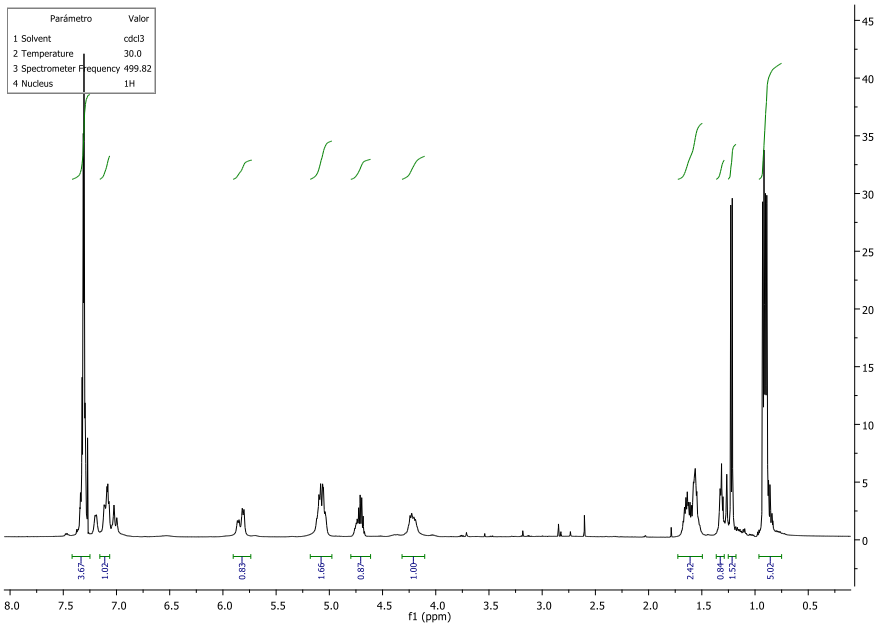
**4.1**

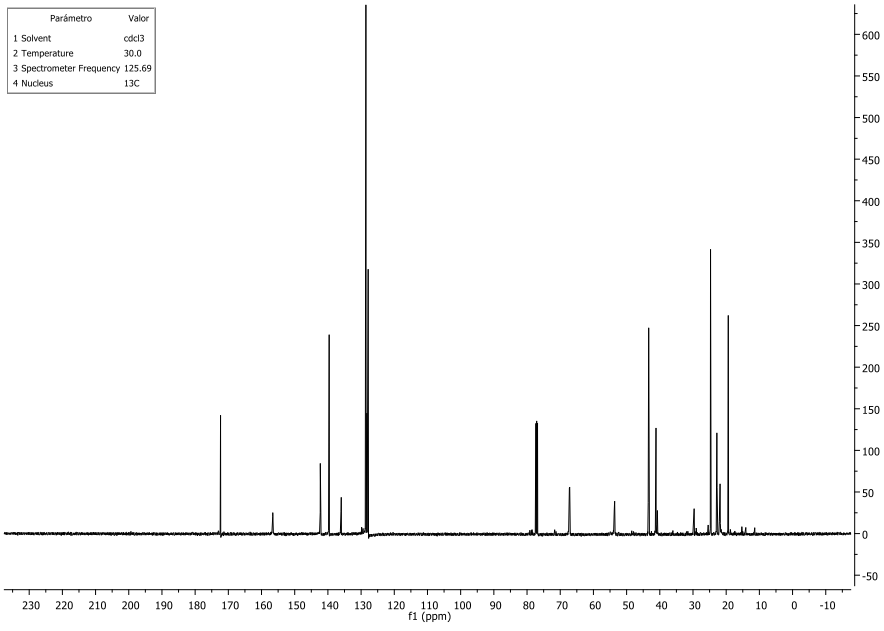

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
6.34
1.63
0.84
0.79
0.91
1.73
1.00
1.82
2.31
f1 (ppm)

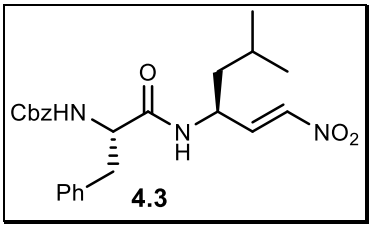

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)


O
Me
CbzHN
N
H
NO2

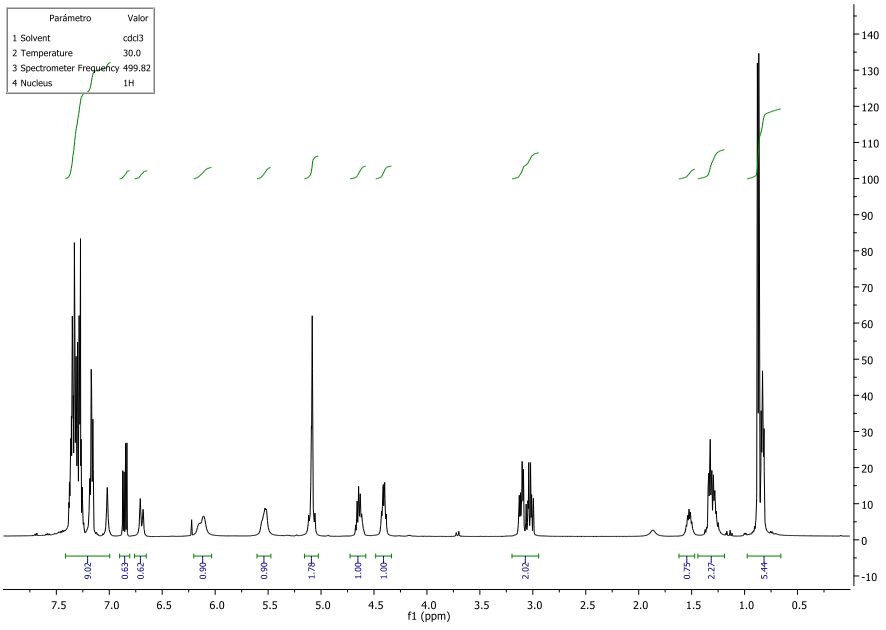
**4.2**

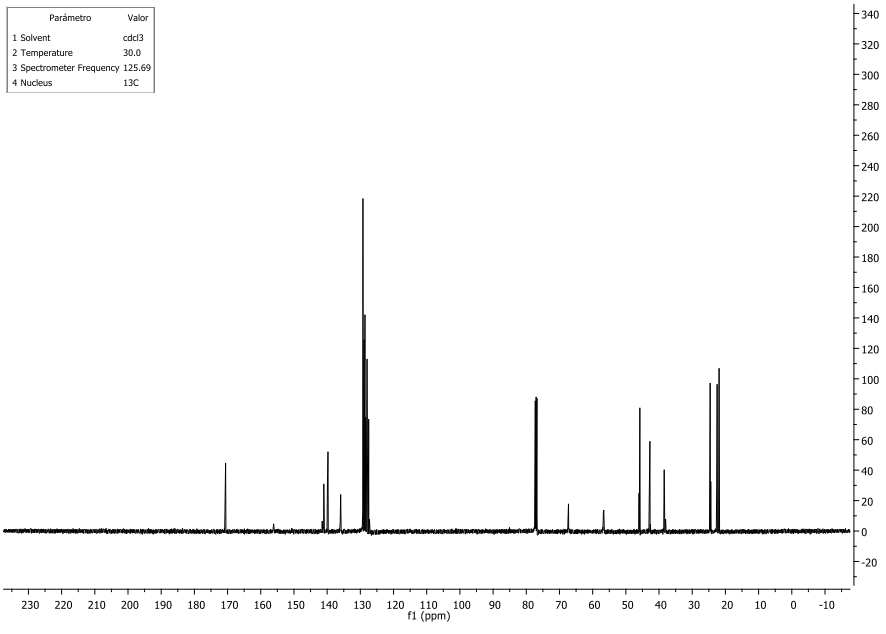

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
3.67
1.02
0.83
1.66
0.87
1.00
2.42
0.84
1.52
5.02
f1 (ppm)

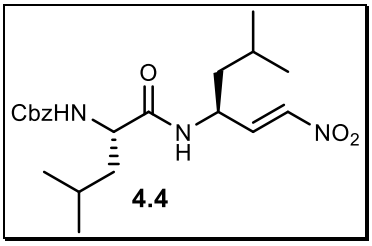

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)


CbzHN
O
N
H
NO2
Ph

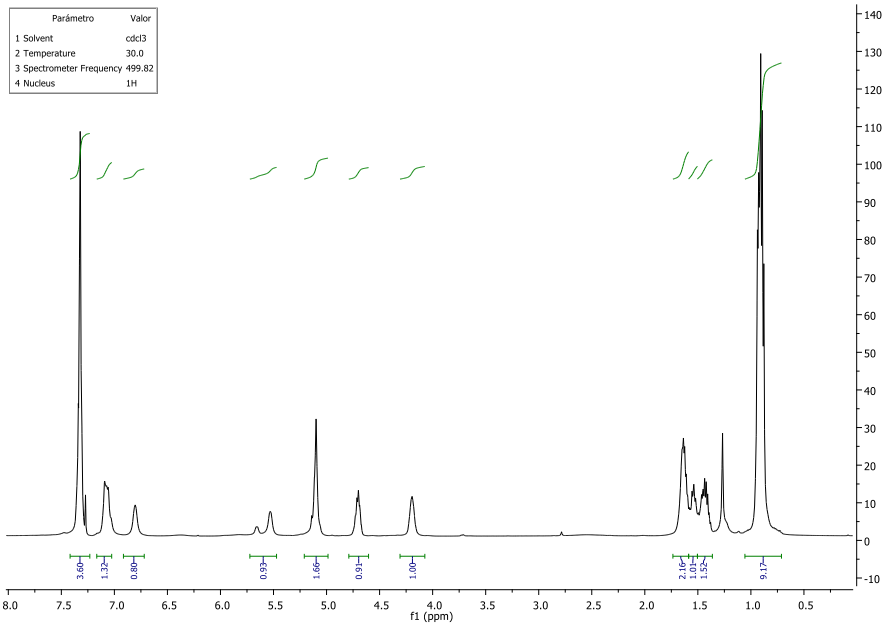
**4.3**

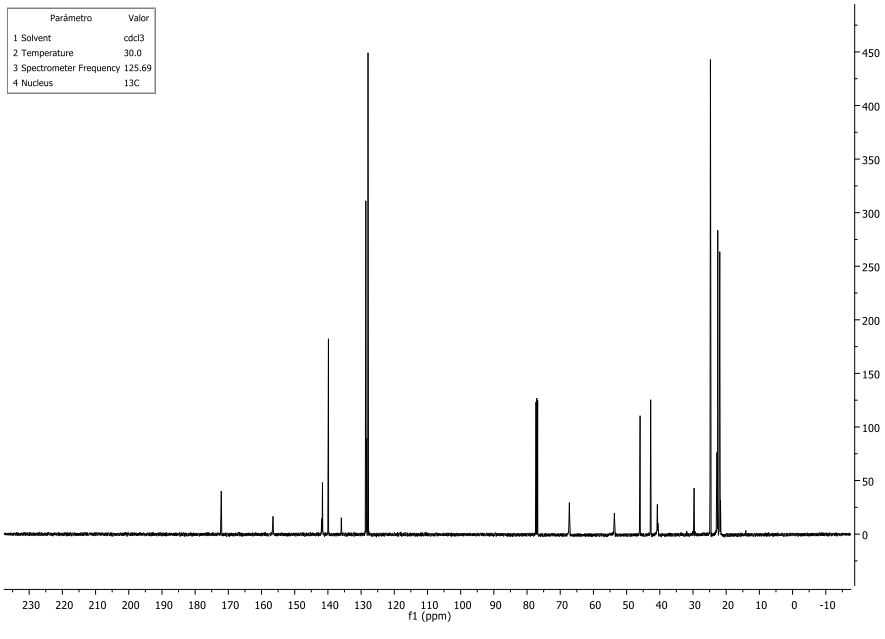

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
9.02
0.63
0.62
0.90
0.90
1.79
1.00
1.00
2.02
0.75
2.27
5.44
f1 (ppm)

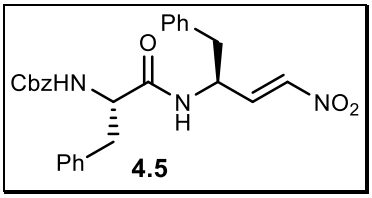

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)


CbzHN
O
N
H
NO2

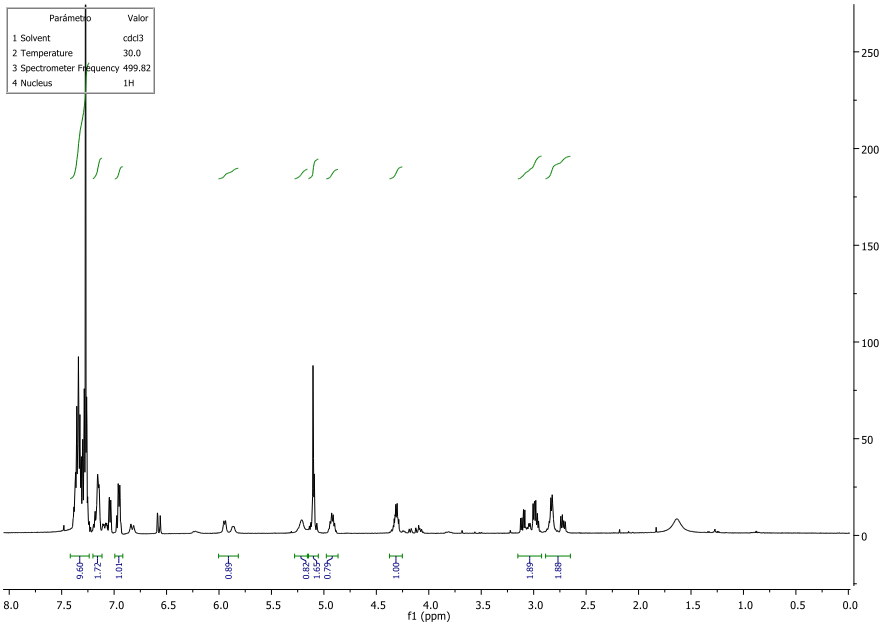
**4.4**

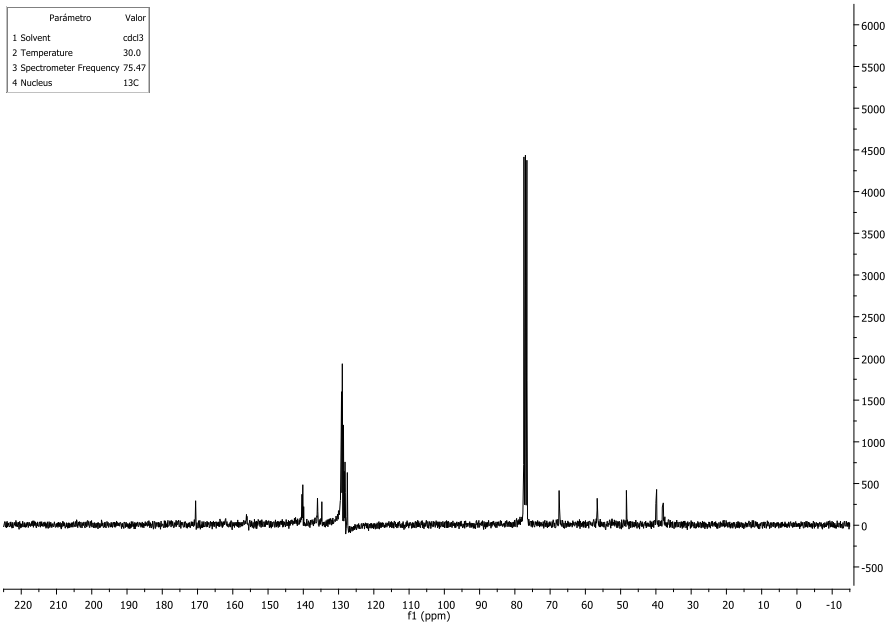

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
3.60
1.32
0.80
0.93
1.66
0.91
1.00
2.16
1.01
1.52
9.17
f1 (ppm)

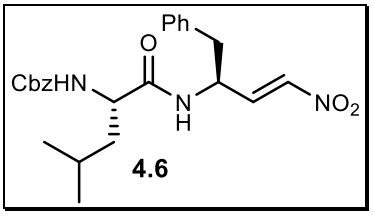

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)


Ph
O
CbzHN
N
H
NO2
Ph

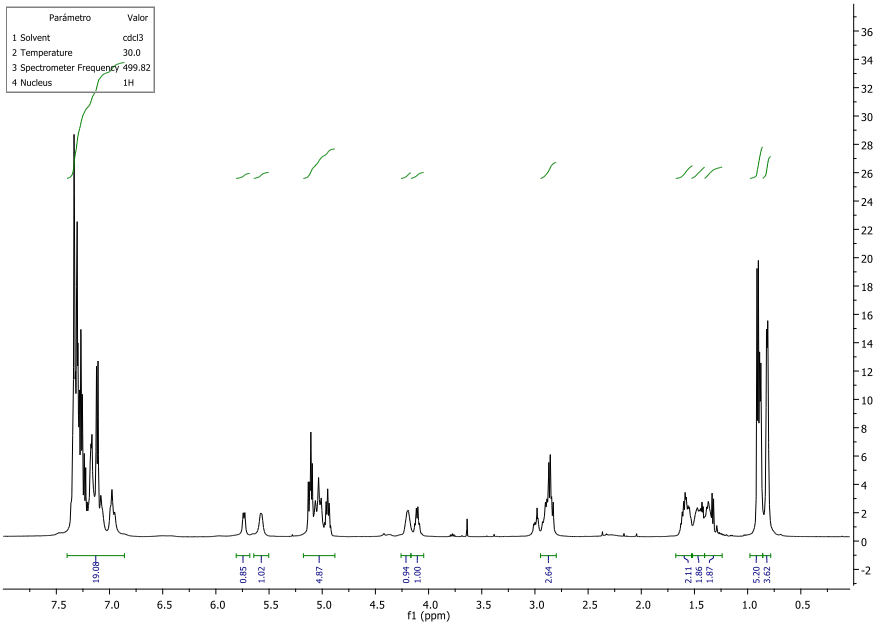
**4.5**

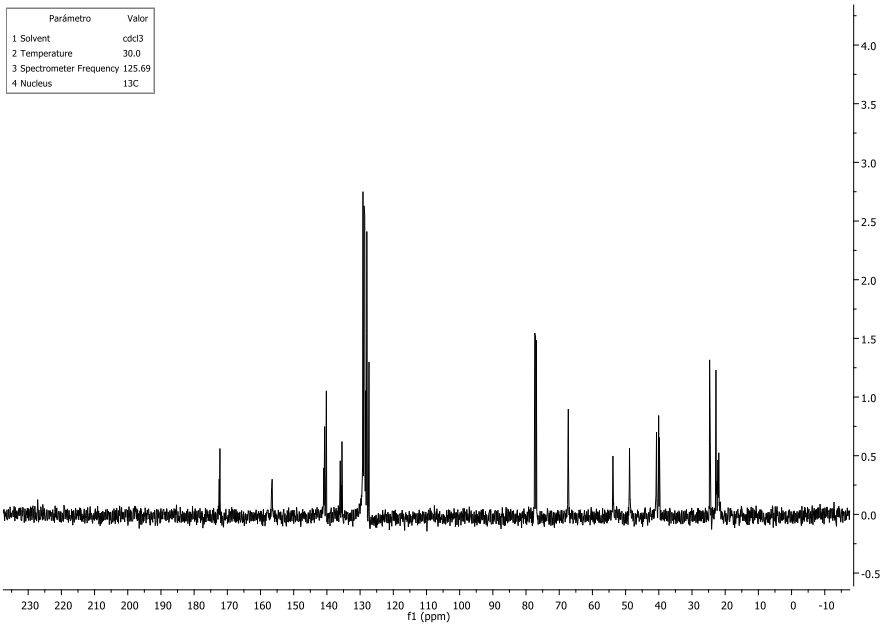

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
9.60
1.72
1.01
0.89
0.82
1.65
0.75
1.00
1.89
1.88
f1 (ppm)

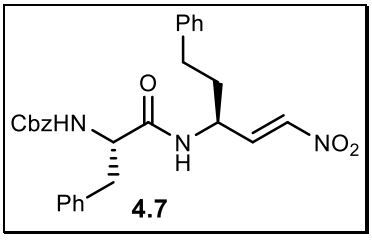

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 75.47
4 Nucleus 13C
f1 (ppm)

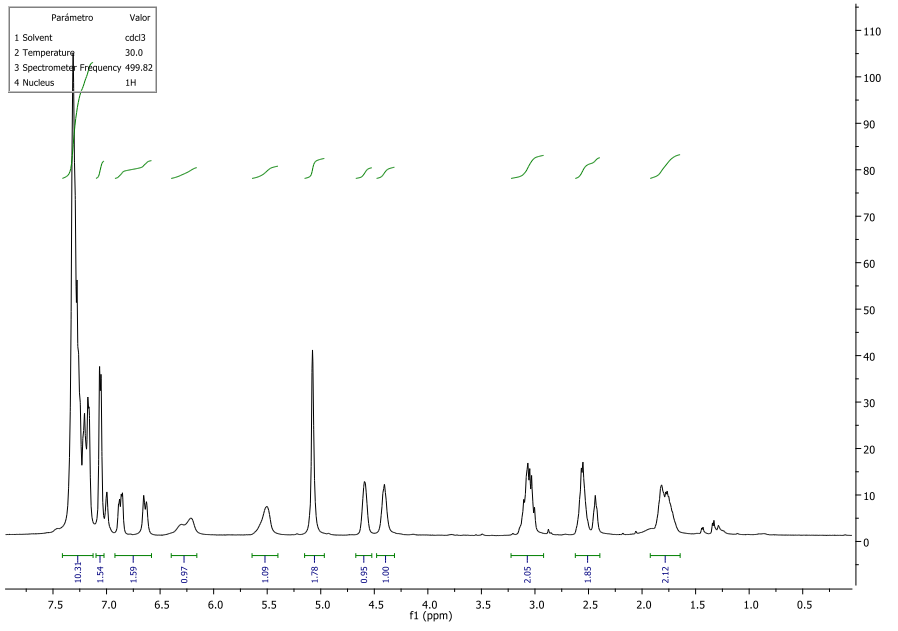

Ph
O
CbzHN
N
H
NO2

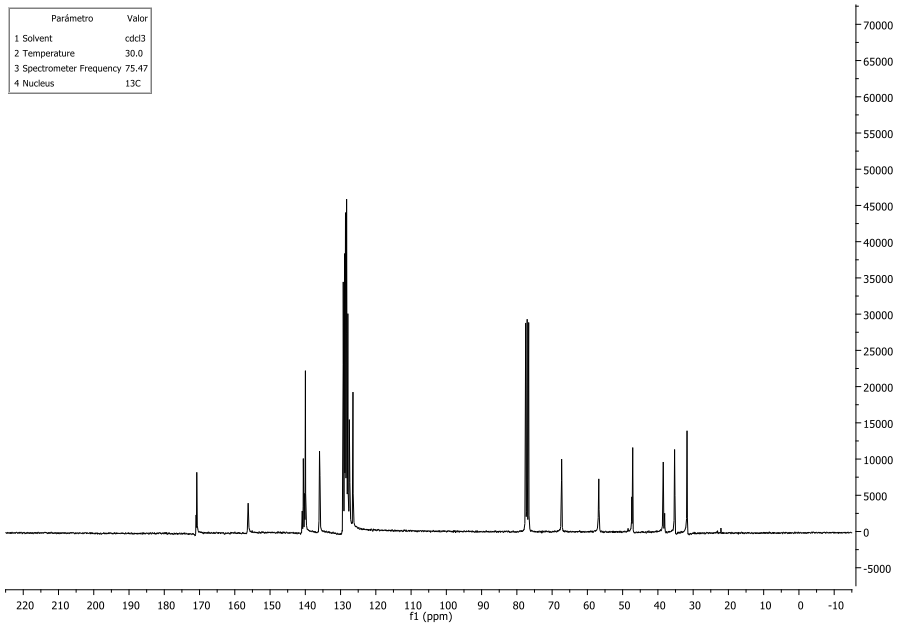
**4.6**

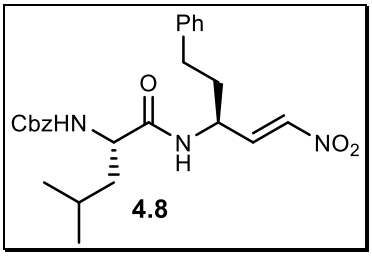

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
19.09
0.85
1.02
4.87
0.94
1.00
2.64
2.11
1.86
1.87
5.20
3.02
f1 (ppm)


Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

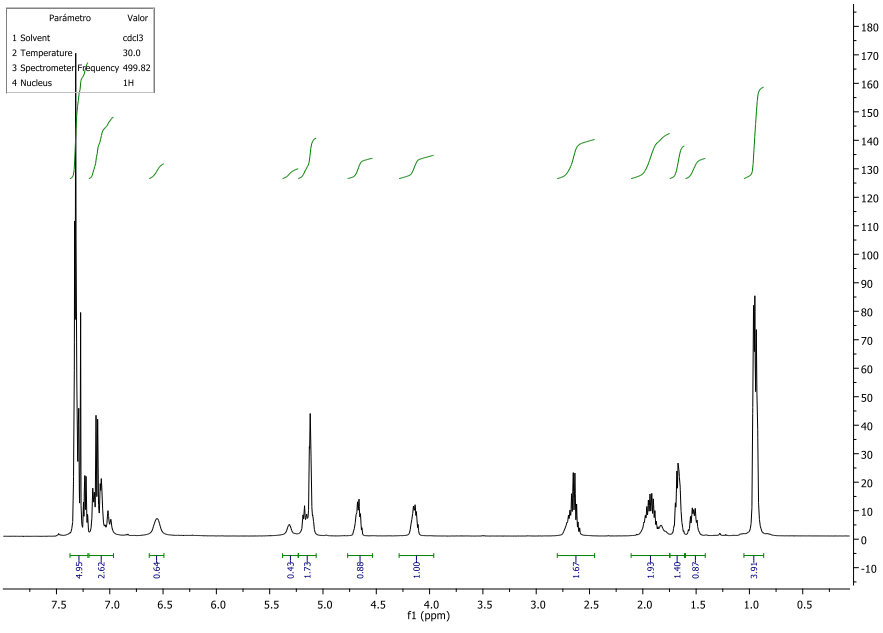

Ph
O
CbzHN
N
H
NO2
Ph
4.7

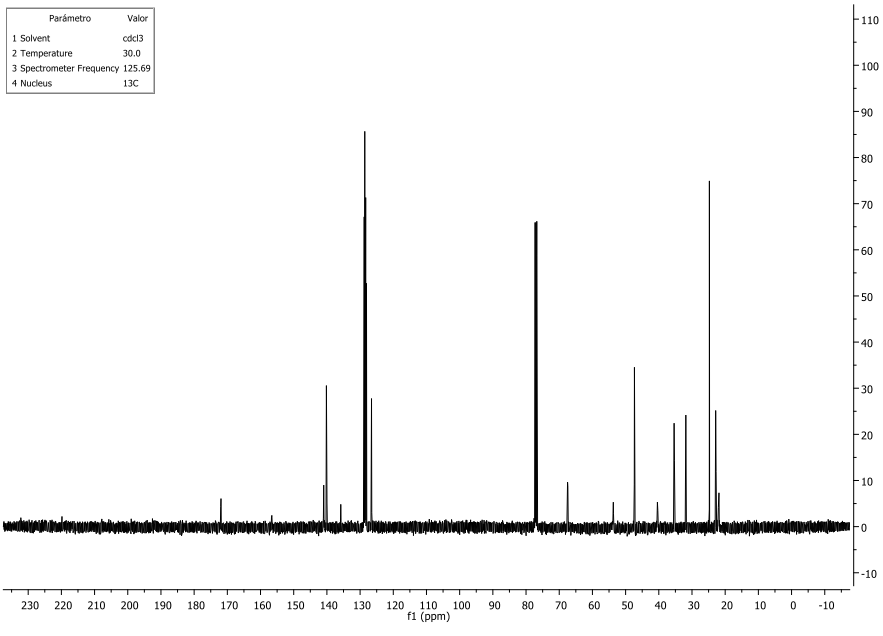

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
10.31
1.54
1.59
0.97
1.09
1.78
0.95
1.00
2.05
1.85
2.12
f1 (ppm)

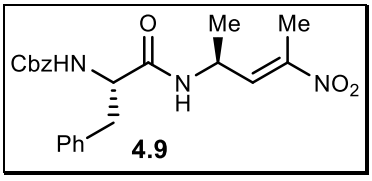

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 75.47
4 Nucleus 13C
f1 (ppm)


Ph
O
CbzHN
N
H
NO2

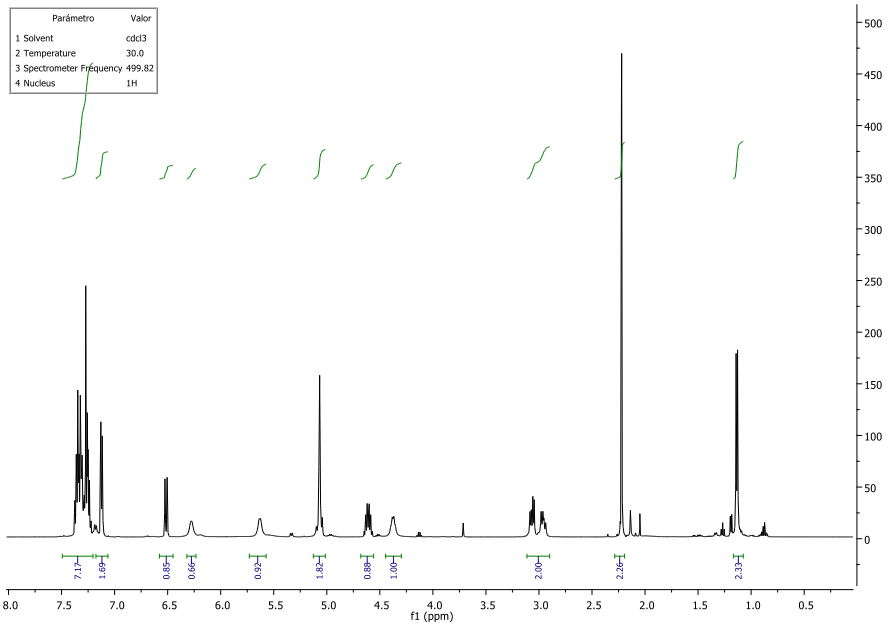
**4.8**

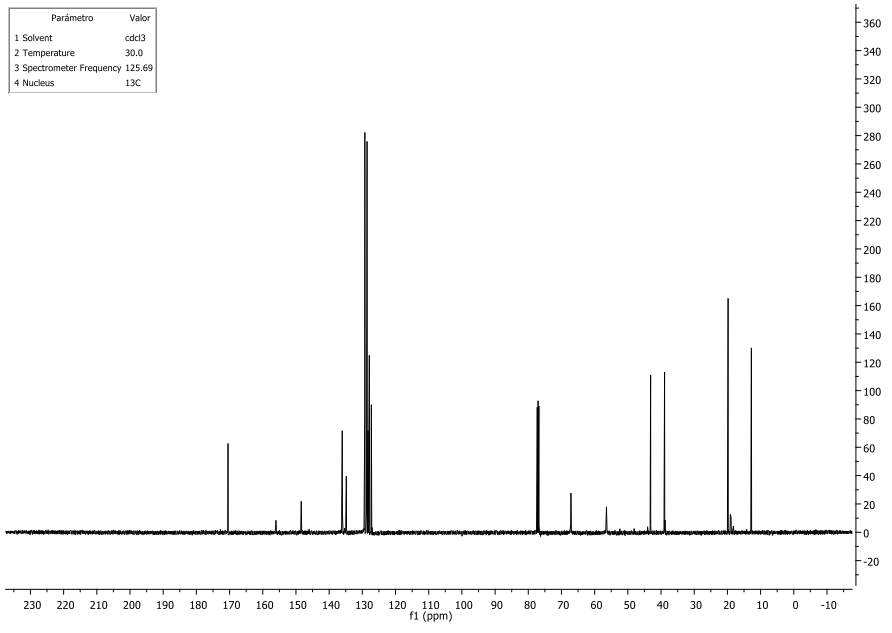

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
4.95
2.62
0.64
0.43
1.79
0.88
1.00
1.67
1.93
1.40
0.87
3.91
f1 (ppm)

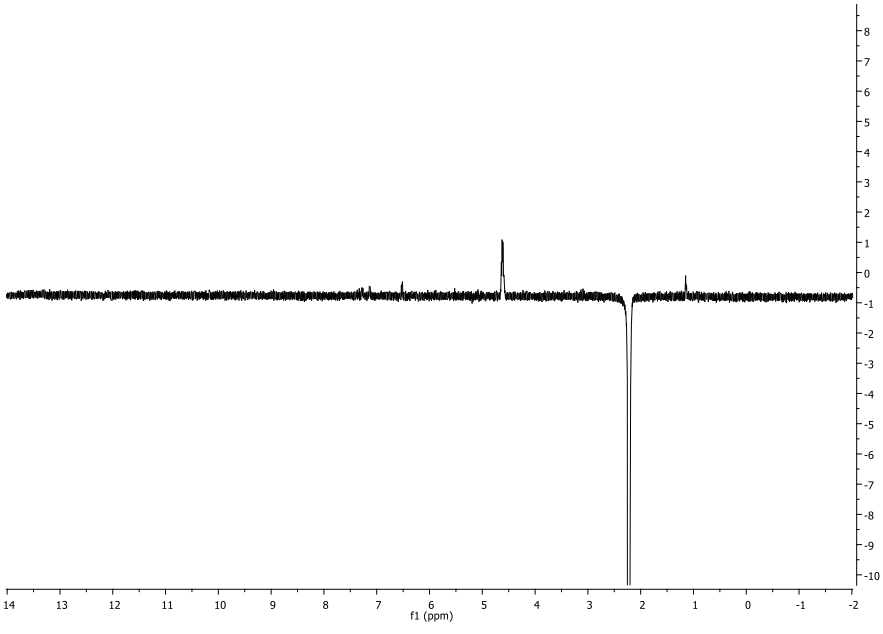

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

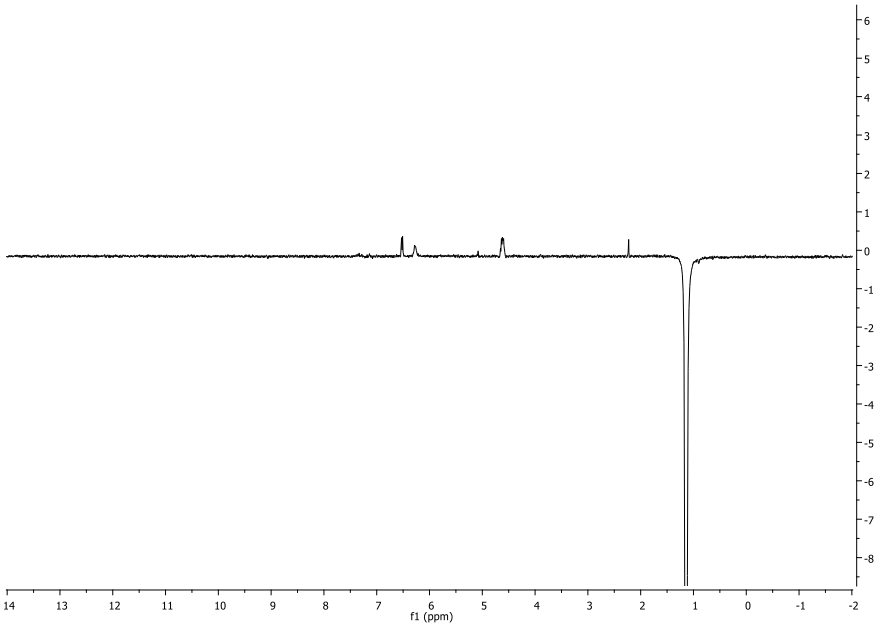

O
Me
Me
CbzHN
N
H
NO2
Ph

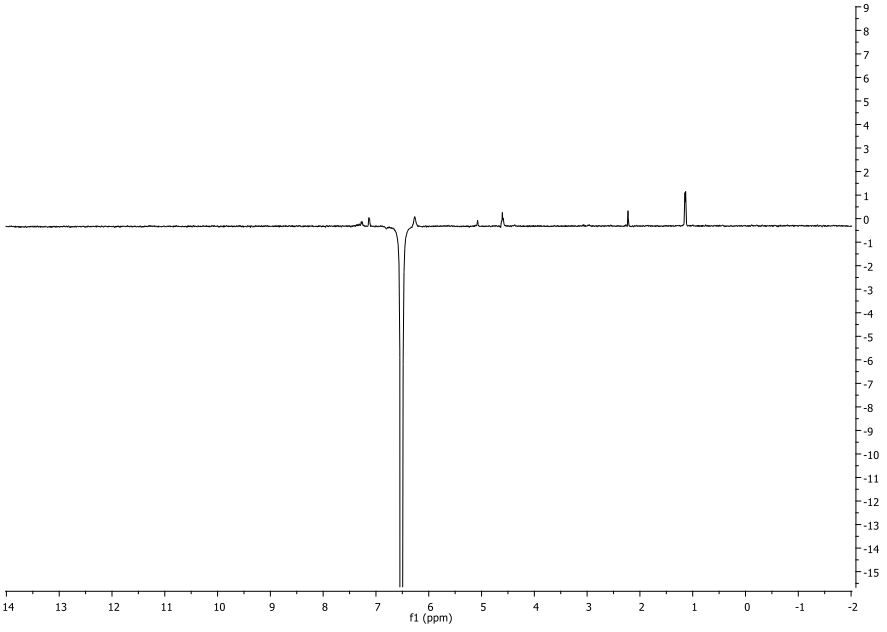
**4.9**

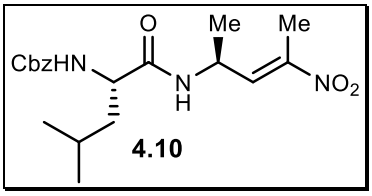

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)


Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

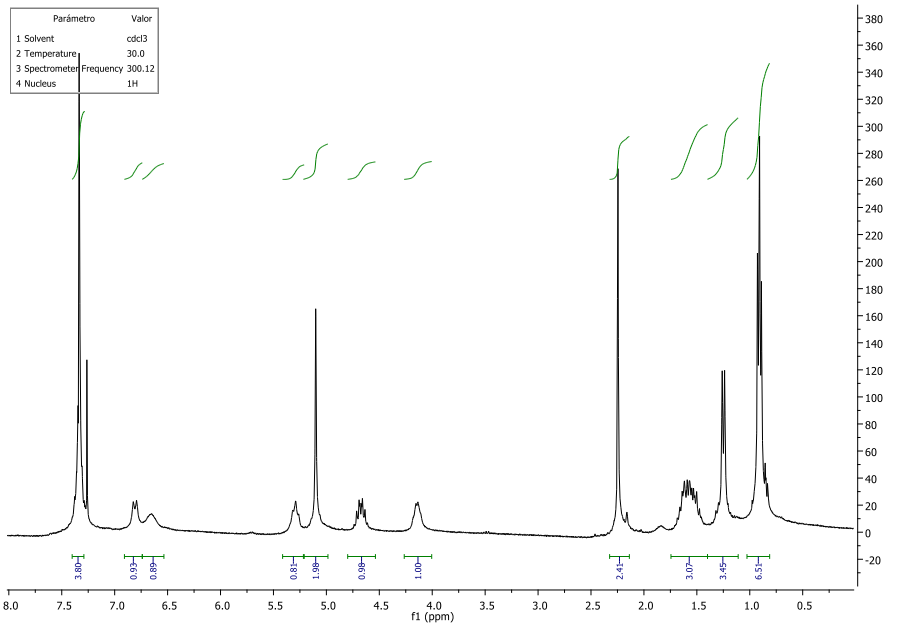

8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

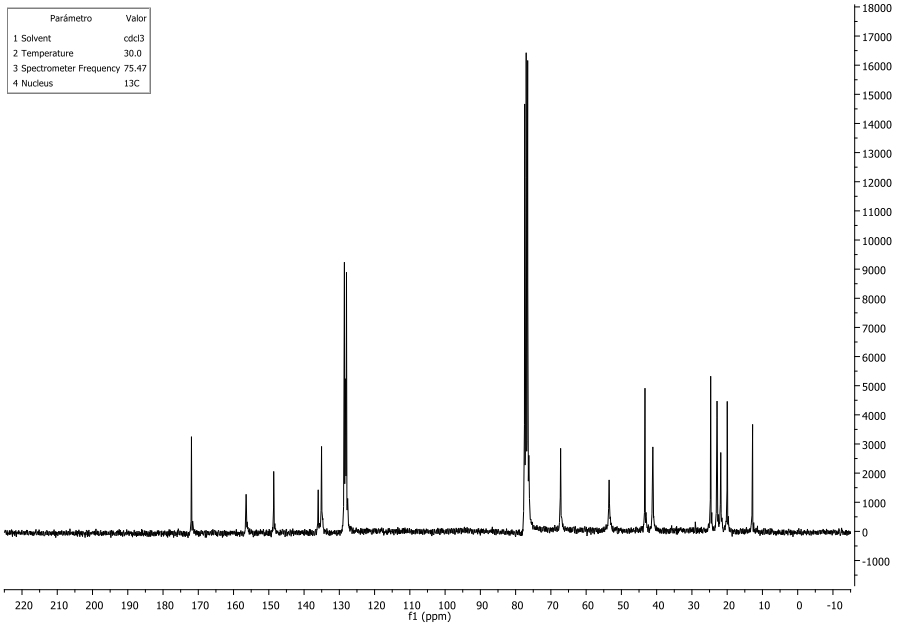

6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

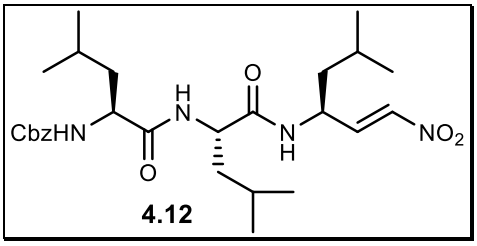

9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
f1 (ppm)


O
Me
Me
CbzHN
N
H
NO2

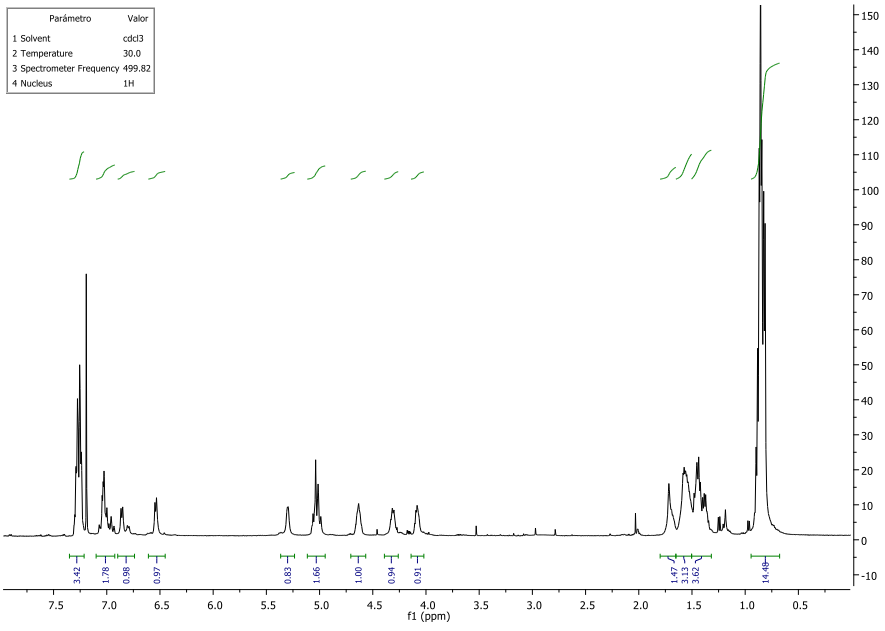
**4.10**

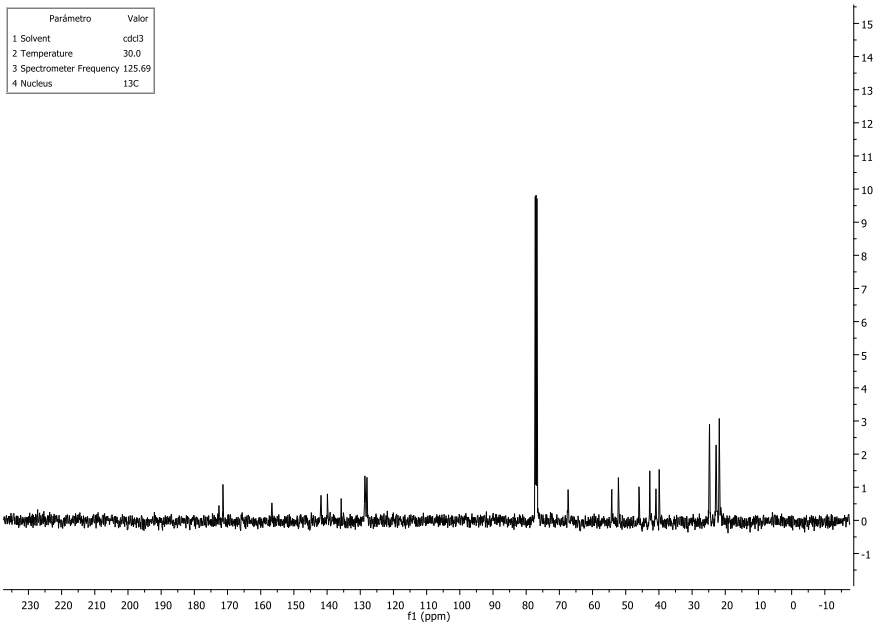

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 300.12
4 Nucleus 1H
3.80
0.99
0.89
0.81
1.98
0.98
1.00
2.41
3.07
3.45
6.51
f1 (ppm)

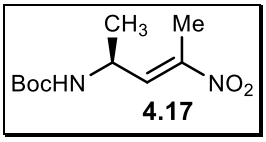

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 75.47
4 Nucleus 13C
f1 (ppm)


CbzHN
H
N
O
O
N
H
NO2

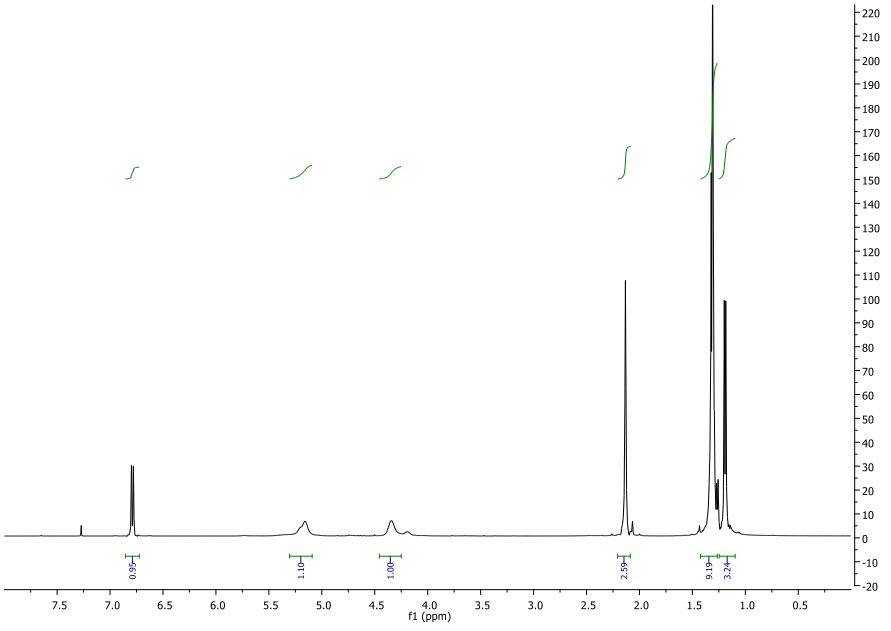
**4.12**

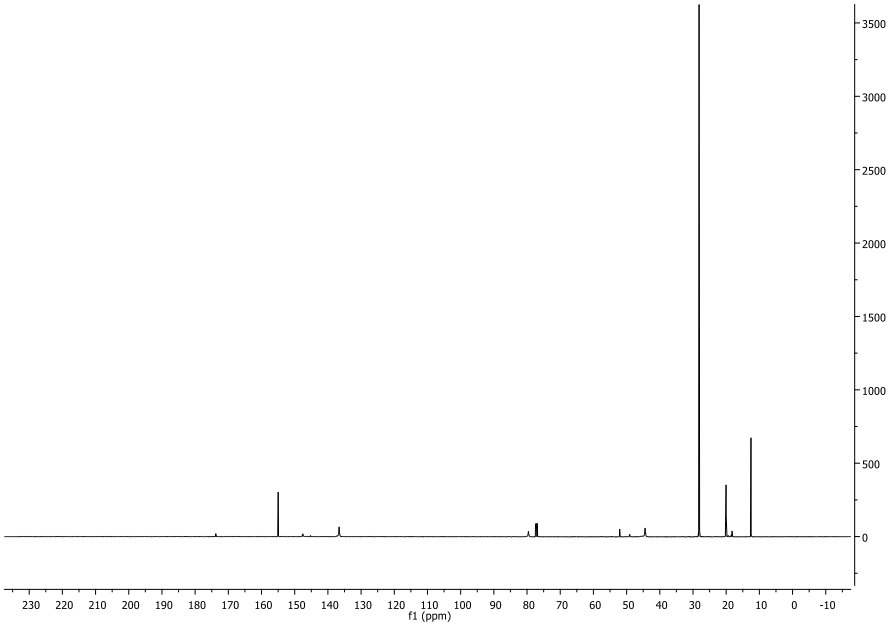

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
3.42
1.78
0.98
0.97
0.83
1.66
1.00
0.94
0.91
1.47
3.13
3.62
14.48
f1 (ppm)

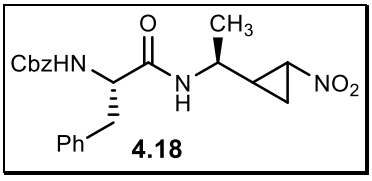

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)



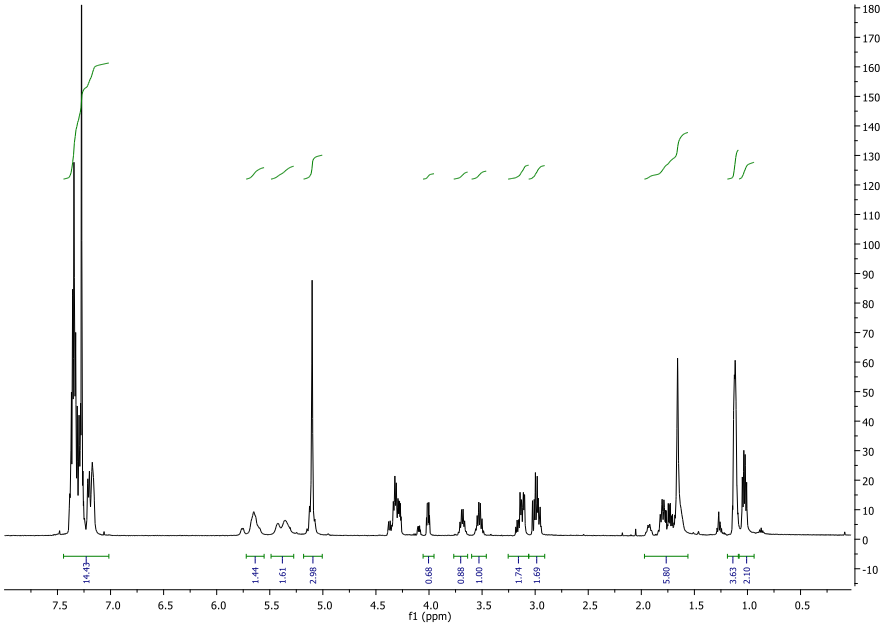

**4.17**

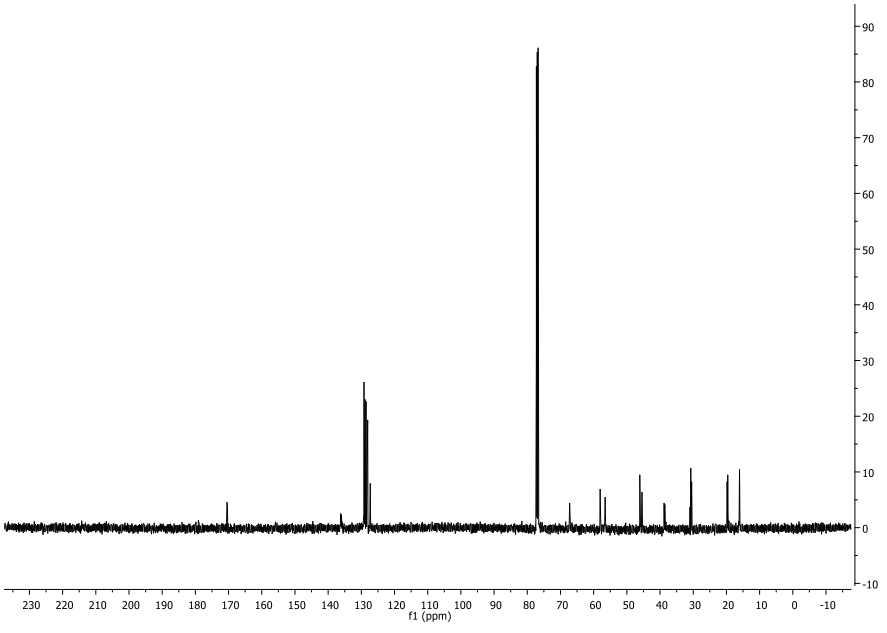


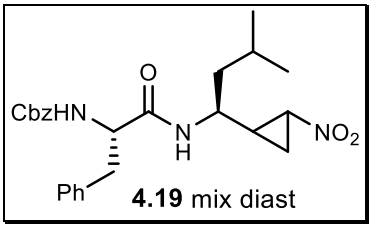




O
CH3
CbzHN
N
H
NO2
Ph

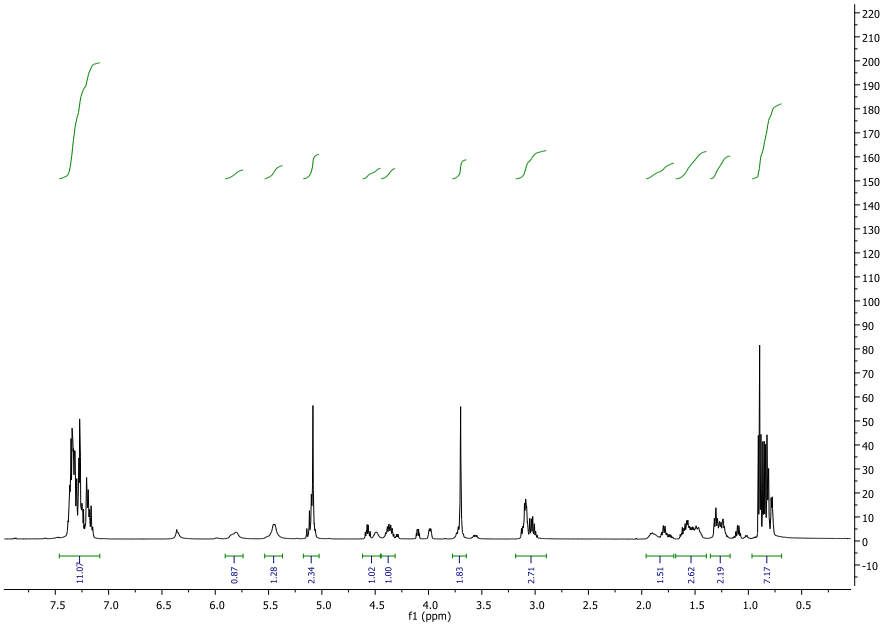
**4.18**

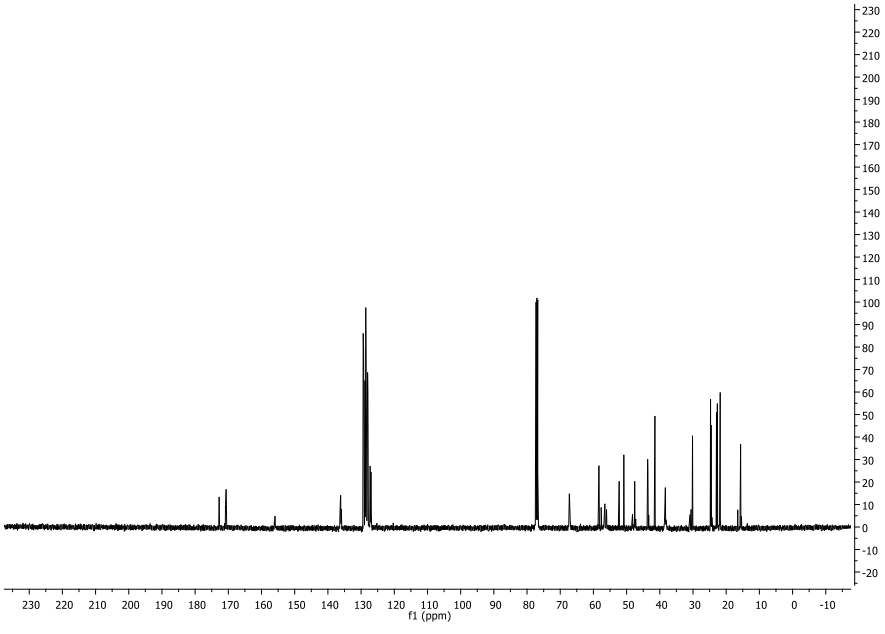

14.43
1.44
1.61
2.98
0.68
0.88
1.00
1.74
1.69
5.80
3.63
2.10
f1 (ppm)

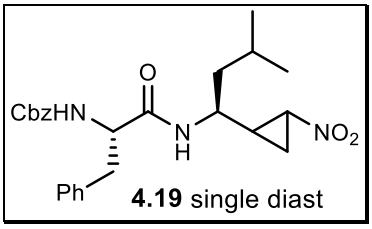

f1 (ppm)



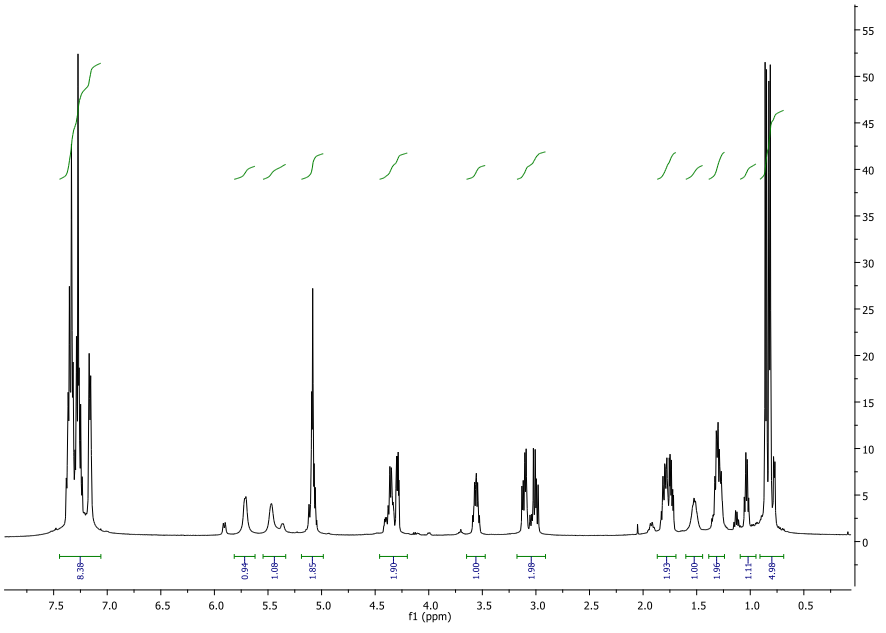

**4.19** mix diast

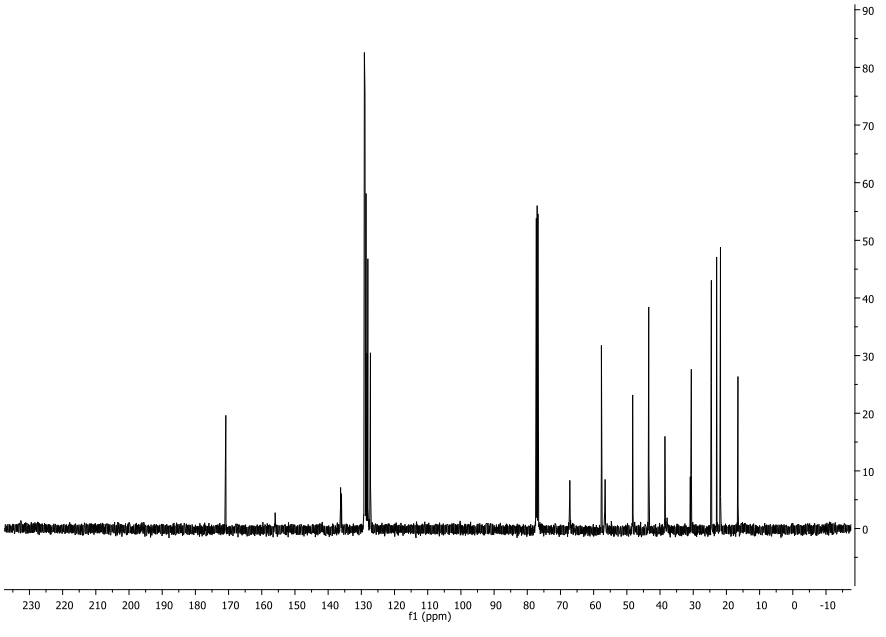


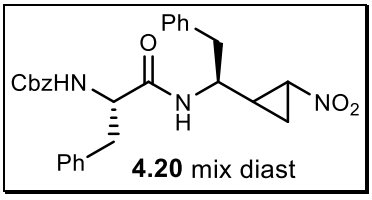





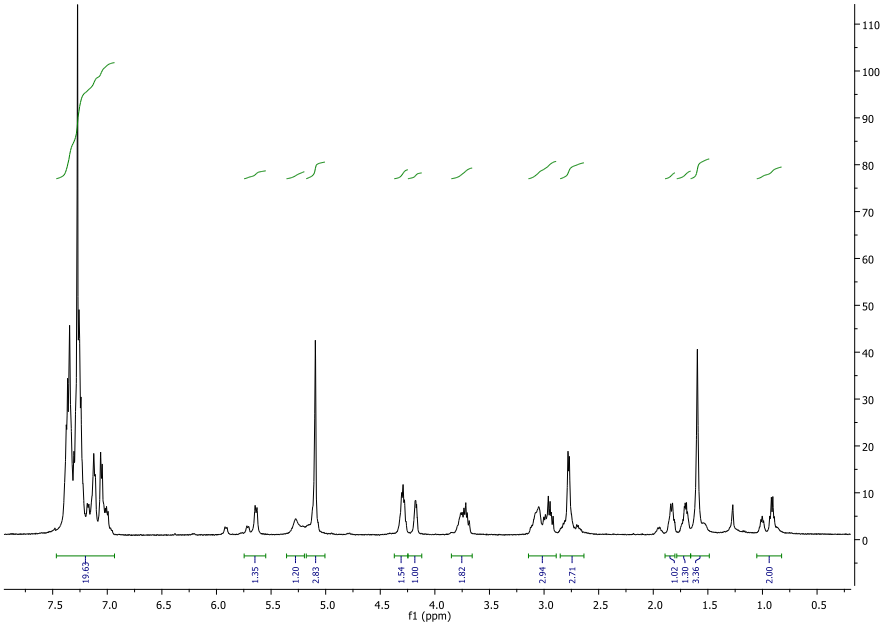

**4.19** single diast

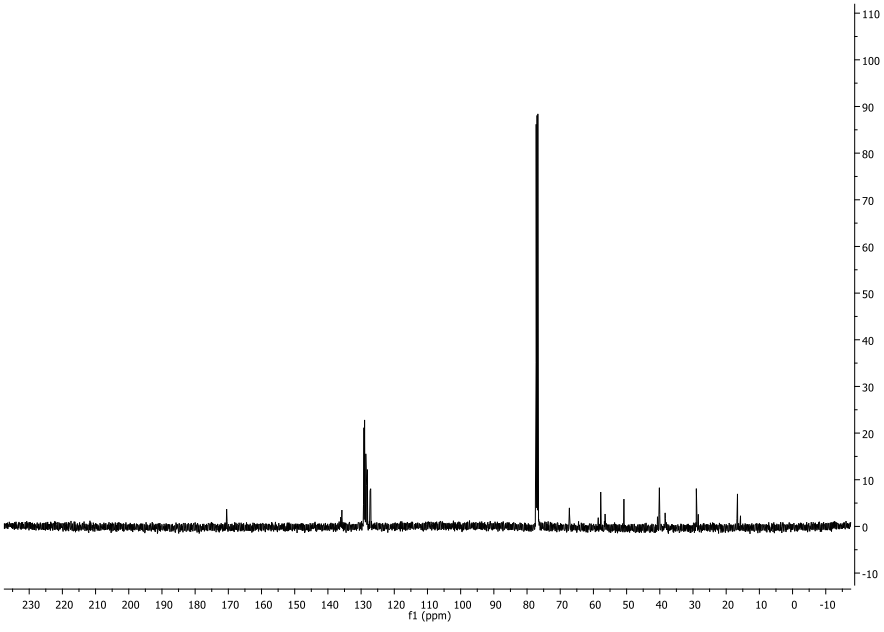


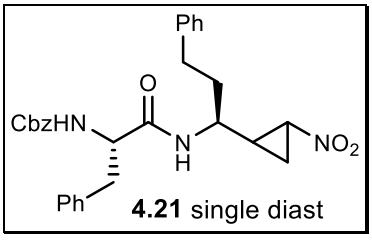





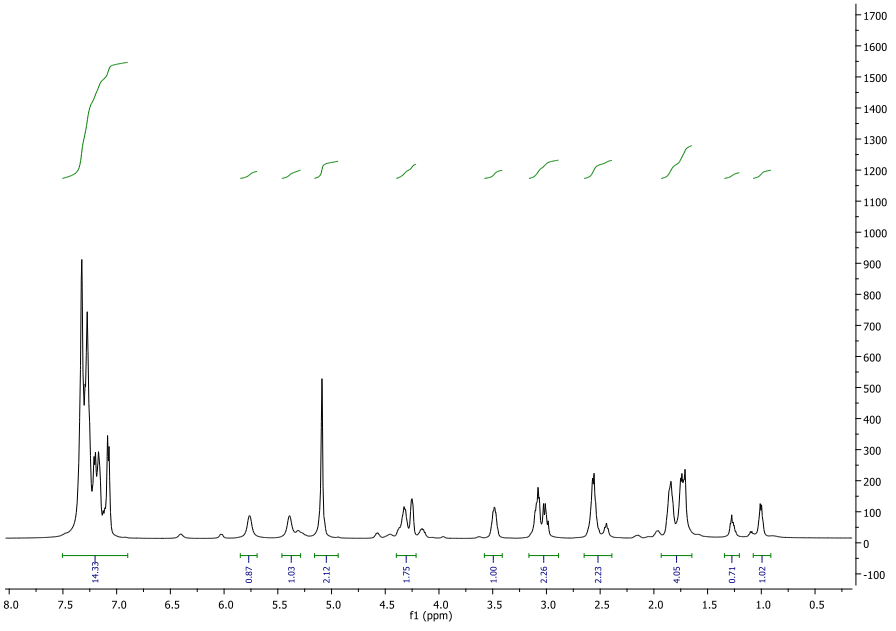

**4.20** mix diast

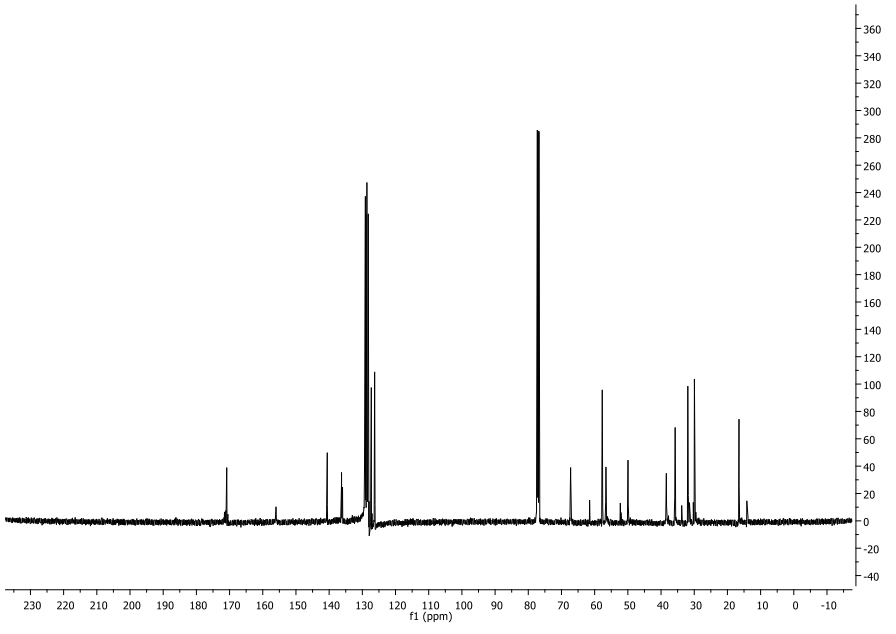


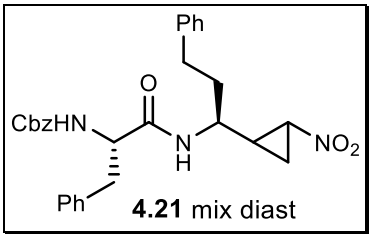





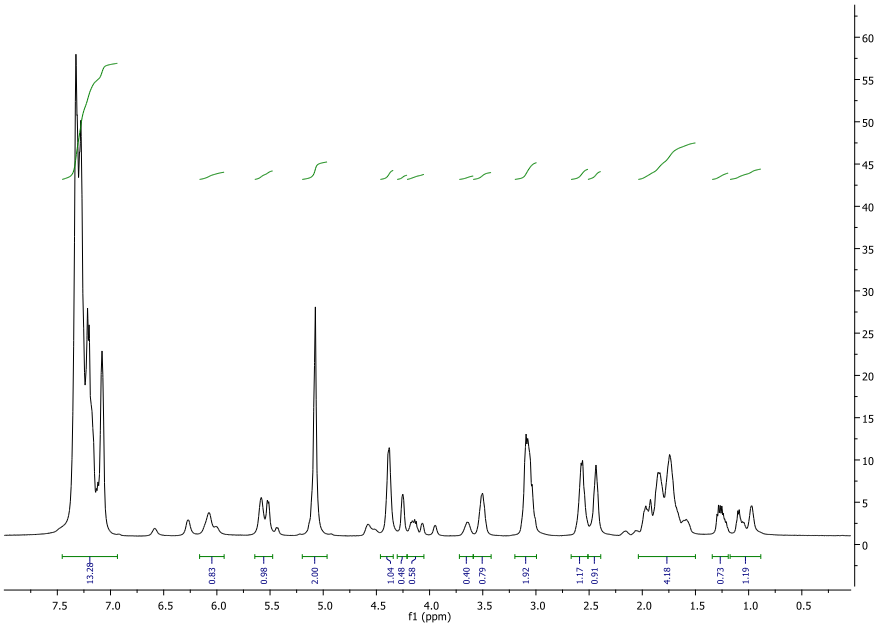

**4.21** single diast

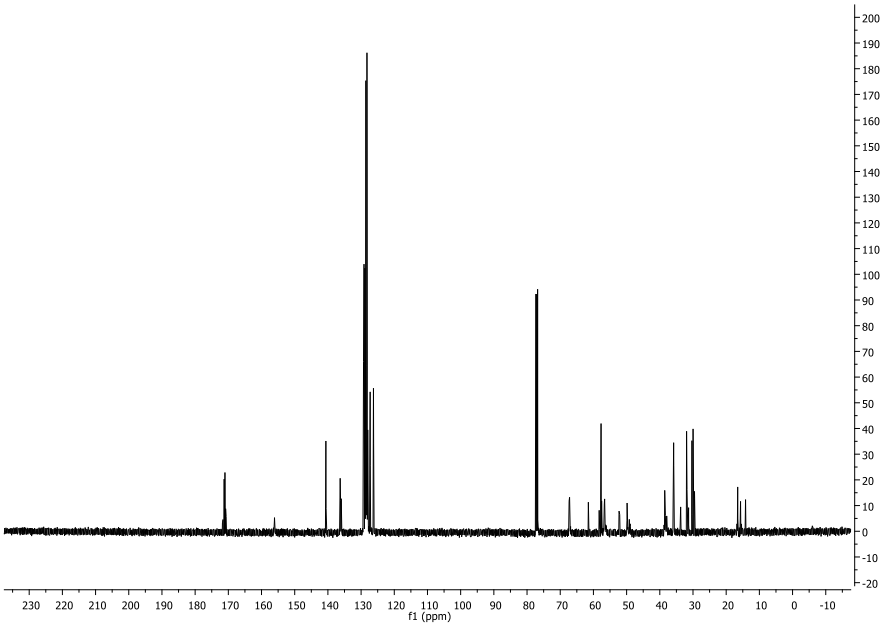


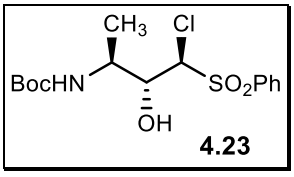





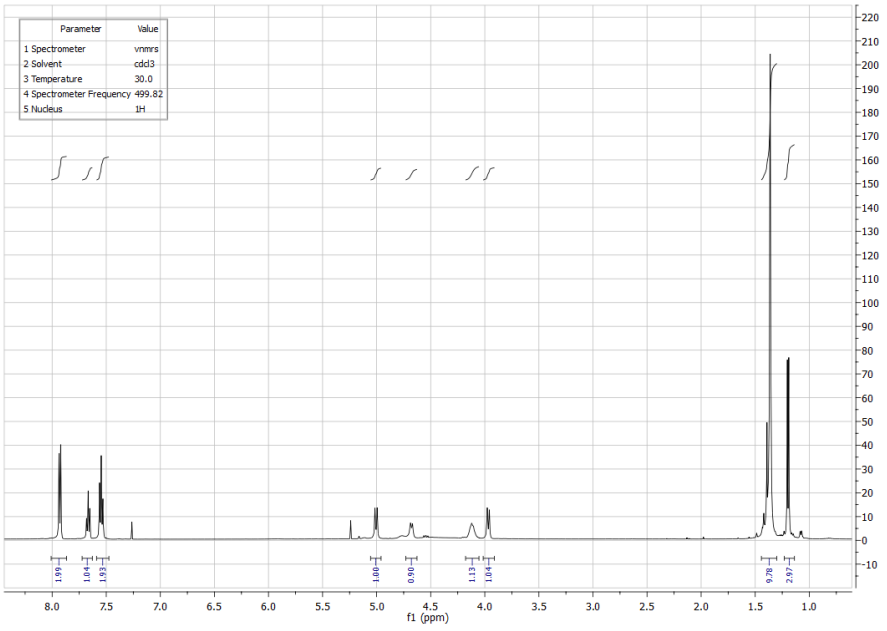

**4.21** mix diast

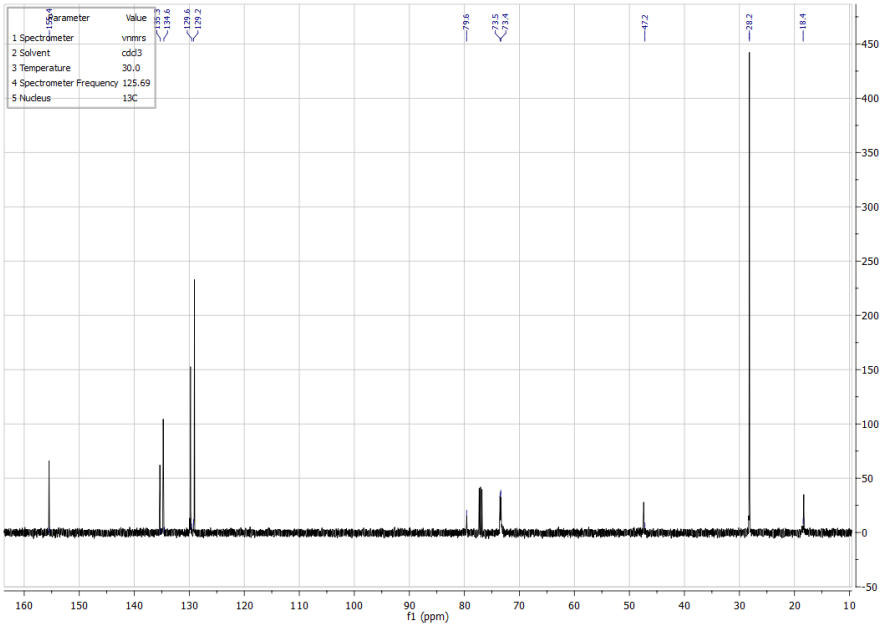


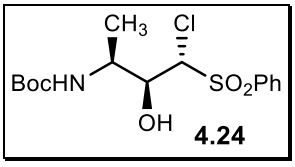




CH3
Cl
BocHN
SO2Ph
OH

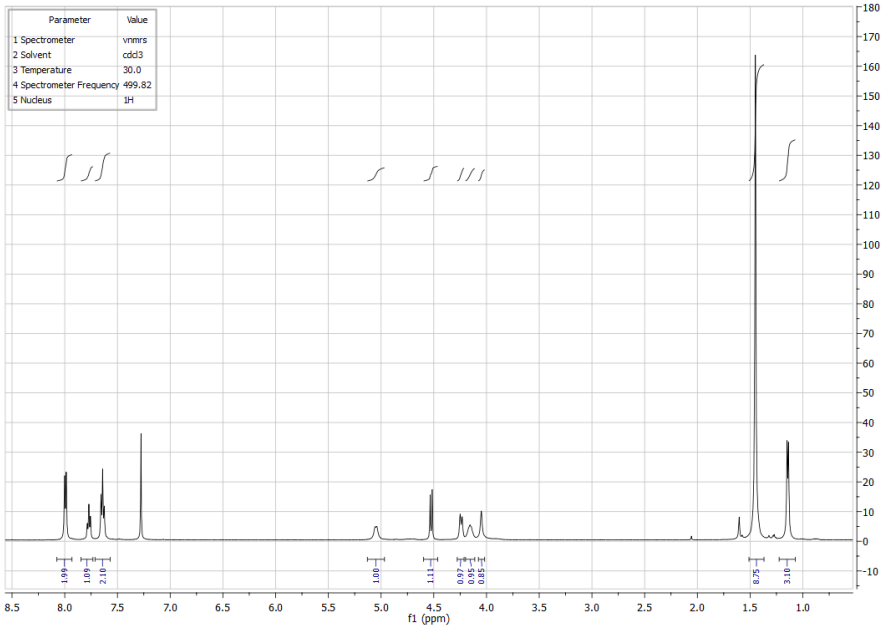
**4.23**

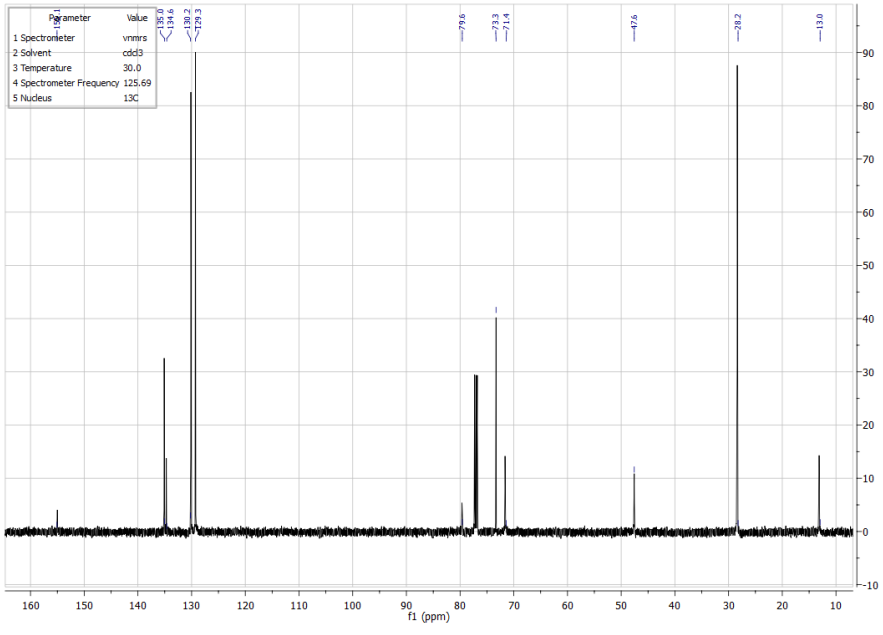

Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 499.82
5 Nucleus 1H
f1 (ppm)

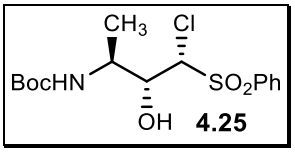

Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 125.69
5 Nucleus 13C
f1 (ppm)


CH3
Cl
BocHN
SO2Ph
OH

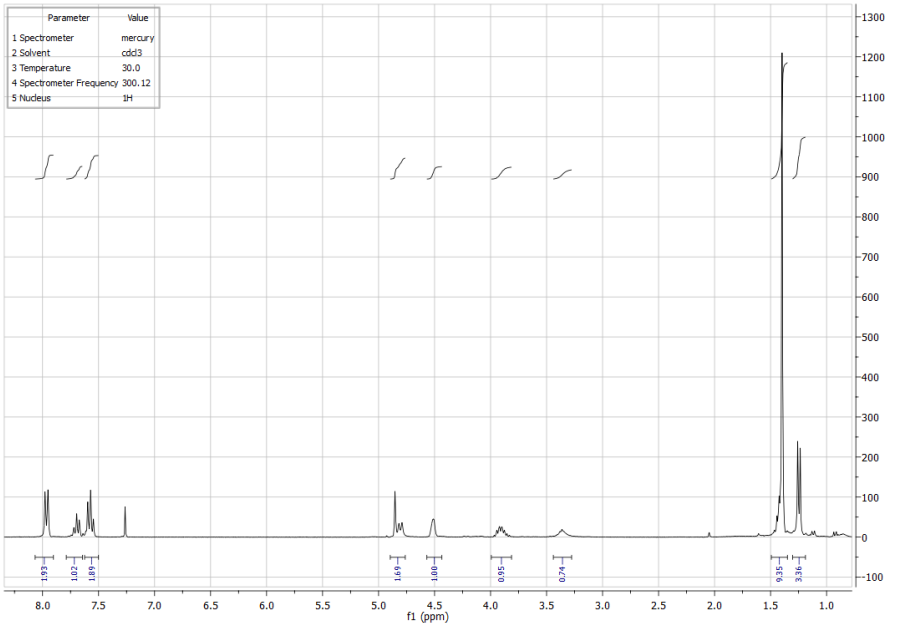
**4.24**

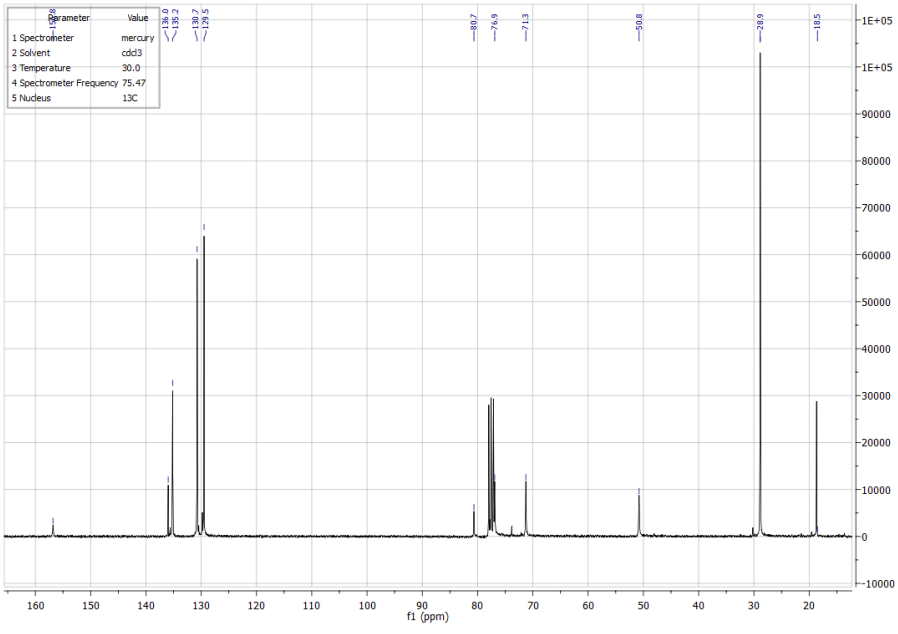

Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 499.82
5 Nucleus 1H
1.99
1.09
2.10
1.00
1.11
0.97
0.95
0.85
8.75
3.10
f1 (ppm)

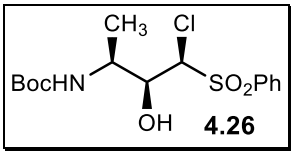

Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 125.69
5 Nucleus 13C
135.0
134.6
130.2
129.3
79.6
73.3
71.4
47.6
28.2
13.0
f1 (ppm)


CH3
Cl
BocHN
SO2Ph
OH

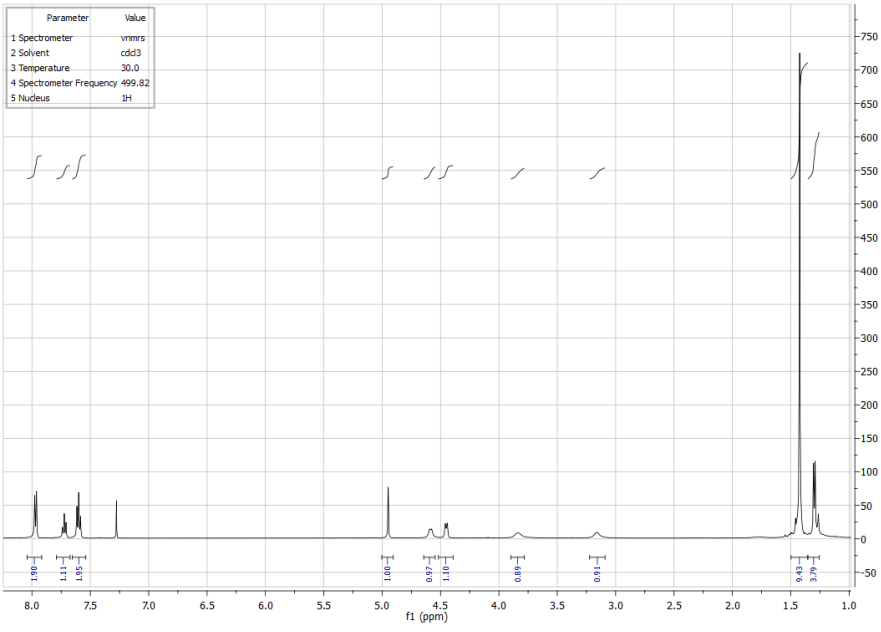
**4.25**

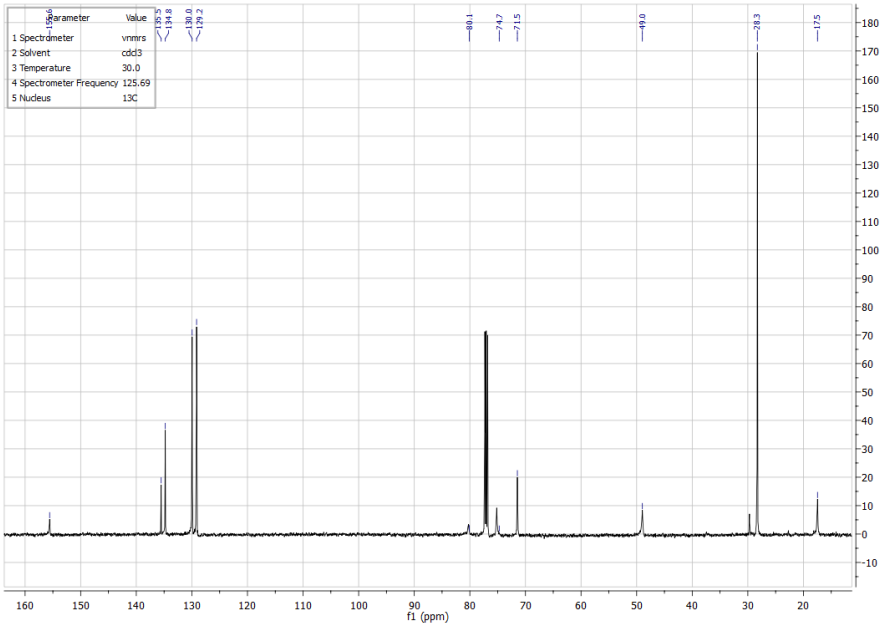

Parameter Value
1 Spectrometer mercury
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 300.12
5 Nucleus 1H
1.93
1.02
1.89
1.69
1.00
0.95
0.74
9.35
5.36
f1 (ppm)

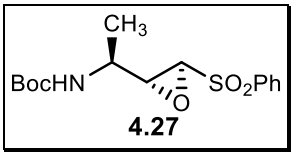

Parameter Value
1 Spectrometer mercury
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 75.47
5 Nucleus 13C
136.0
135.2
130.7
129.5
80.7
76.9
71.3
59.8
28.9
18.5
f1 (ppm)


CH3
Cl
BocHN
SO2Ph
OH

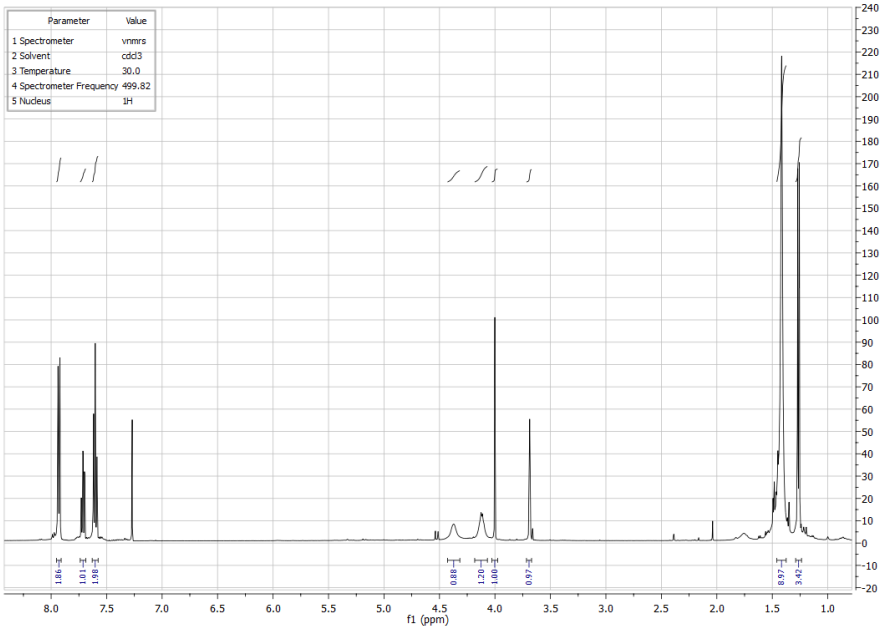
**4.26**

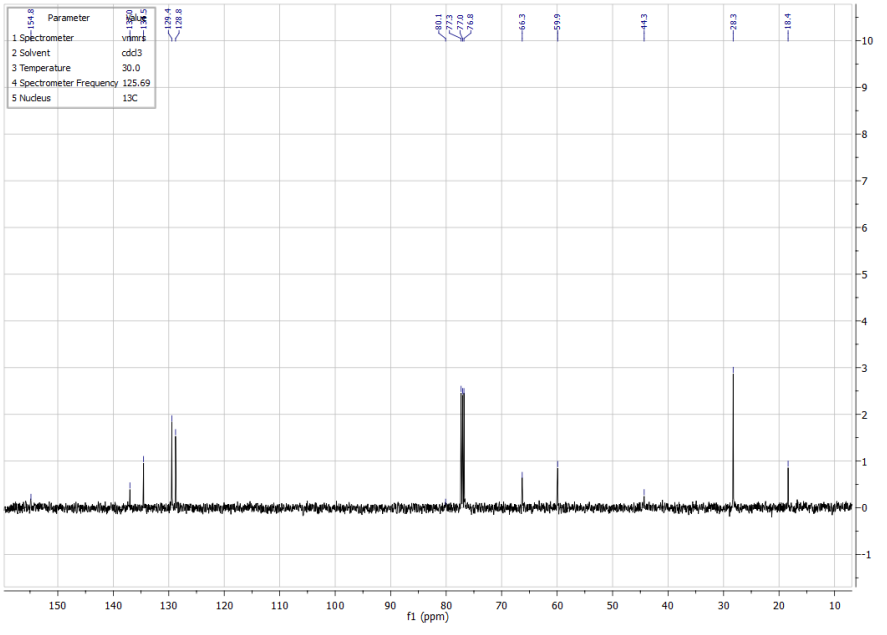

Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 499.82
5 Nucleus 1H
1.90
1.11
1.95
1.00
0.97
1.10
0.89
0.91
9.43
3.70
f1 (ppm)

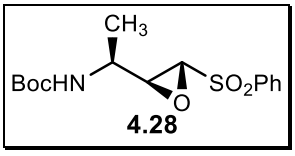

Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 125.69
5 Nucleus 13C
135.5
134.8
130.0
129.2
80.1
74.7
71.5
49.0
28.3
17.5
f1 (ppm)


CH3
BocHN
SO2Ph
O

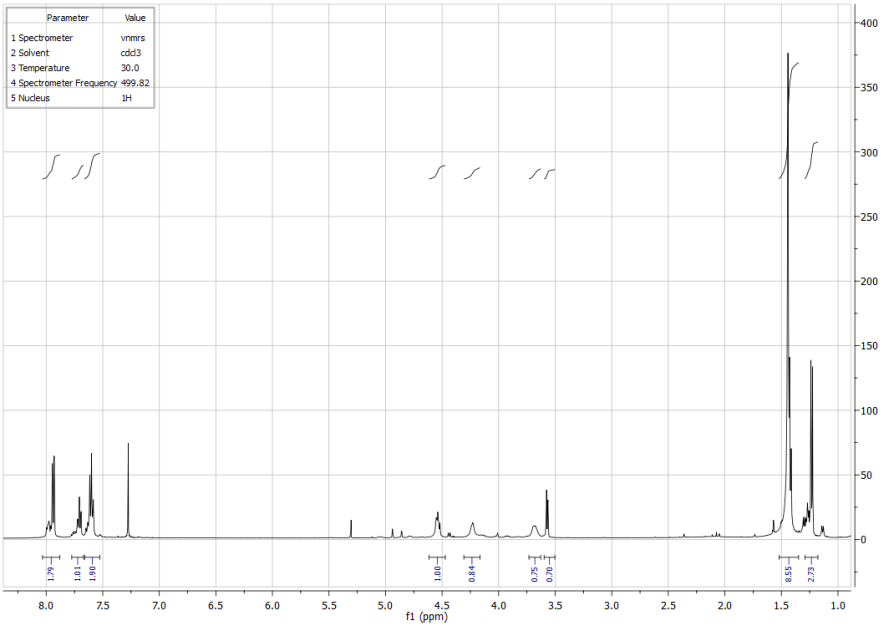
**4.27**

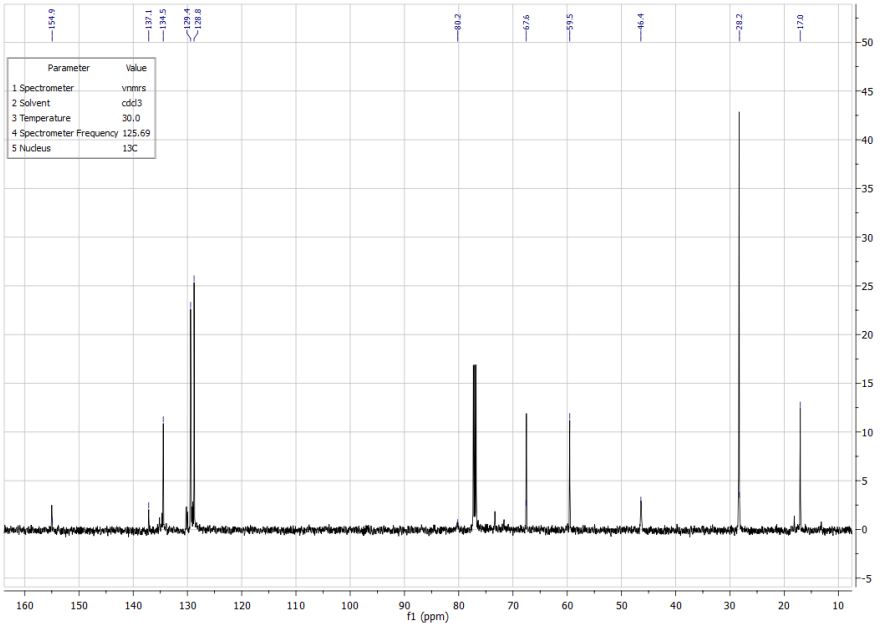

Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 499.82
5 Nucleus 1H
1.86
1.01
1.98
0.88
1.20
1.00
0.97
8.97
3.42
f1 (ppm)

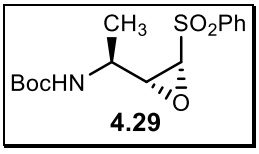

Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 125.69
5 Nucleus 13C
154.8
129.4
128.8
80.1
77.3
77.0
76.8
66.3
59.9
14.3
28.3
18.4
f1 (ppm)


CH3
BocHN
SO2Ph
O

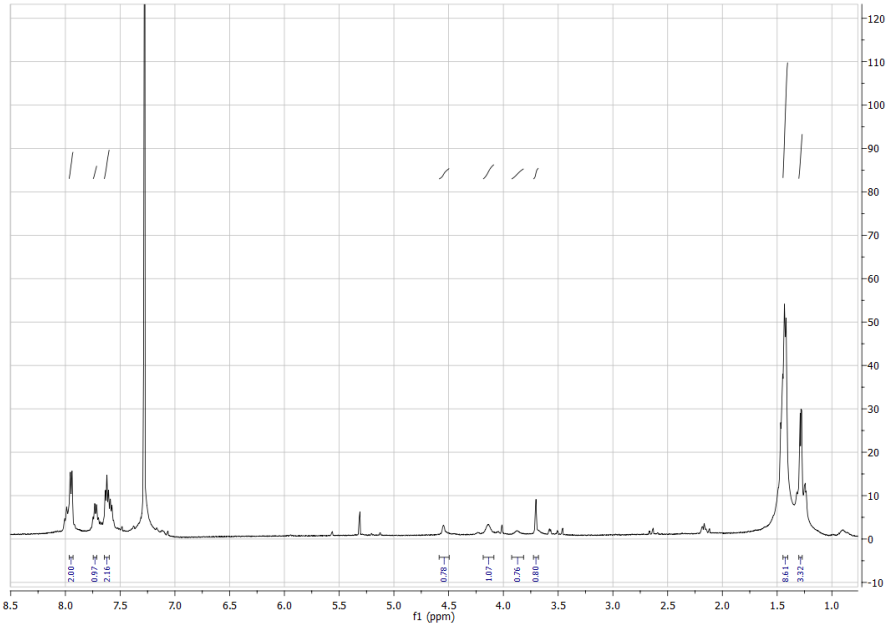
**4.28**

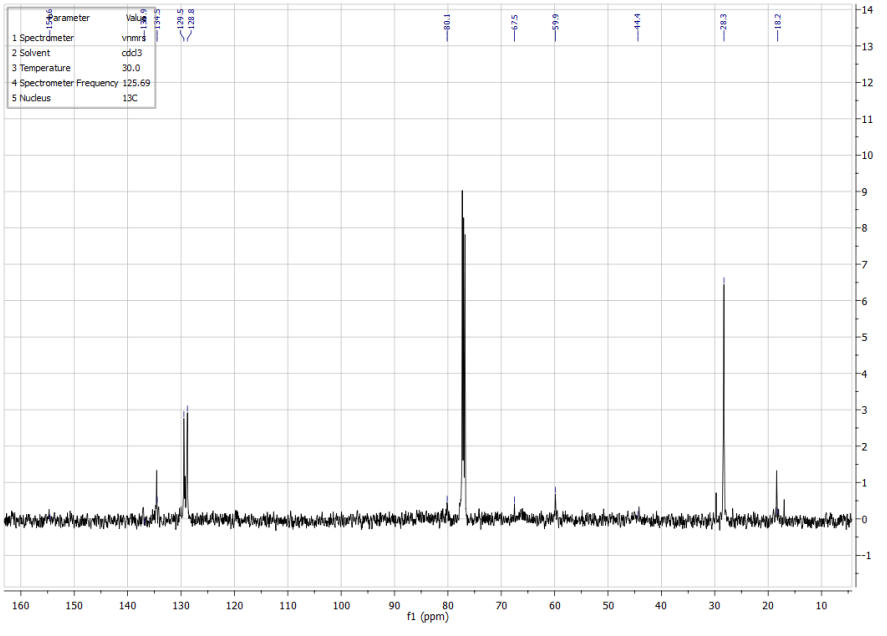

Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 499.82
5 Nucleus 1H
1.79
1.01
1.90
1.00
0.84
0.75
0.70
8.55
2.73
f1 (ppm)

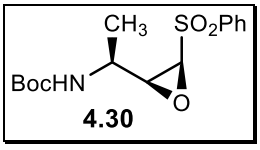

154.9
137.1
134.5
129.4
128.8
80.2
67.6
59.5
46.4
28.2
17.0
Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 125.69
5 Nucleus 13C
f1 (ppm)


CH3
SO2Ph
BocHN
O

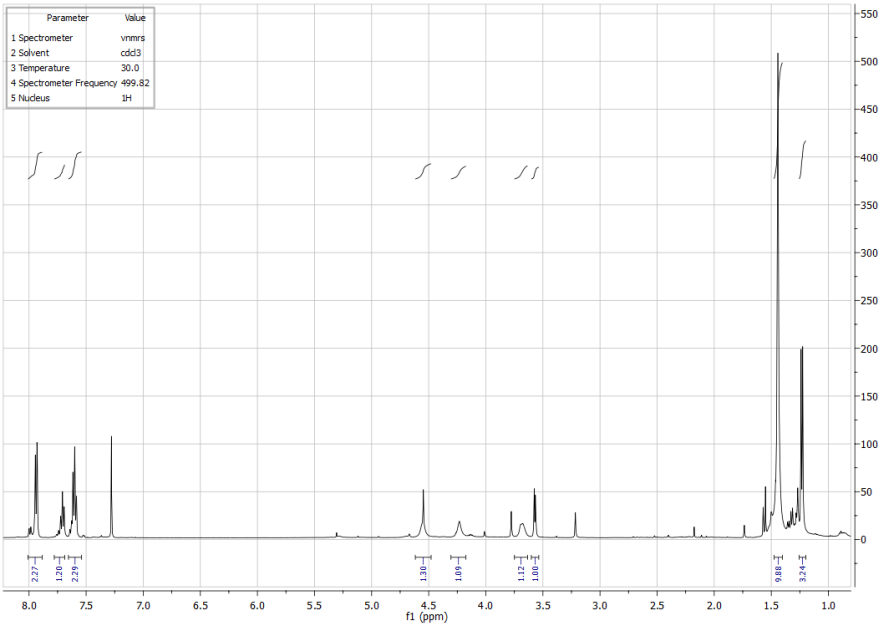
**4.29**

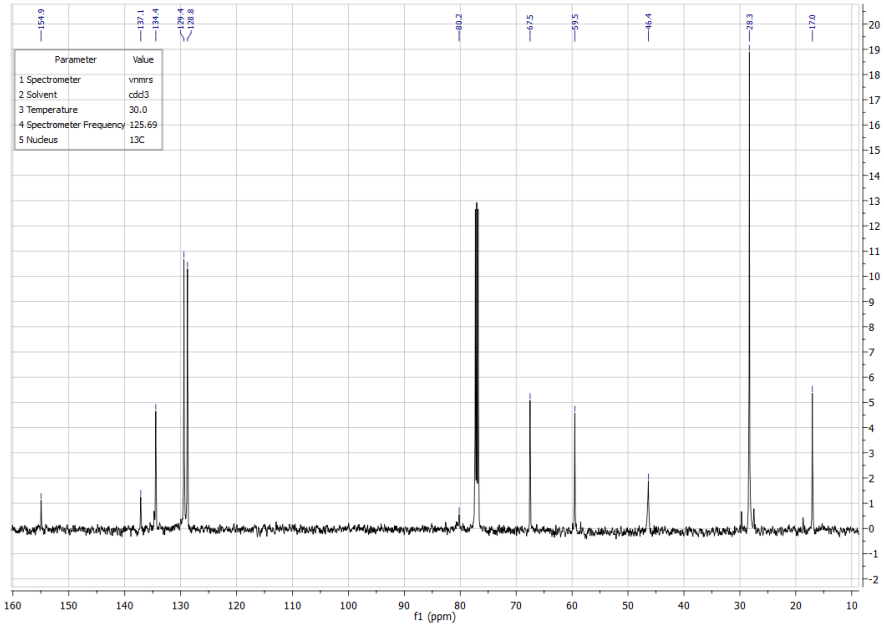

f1 (ppm)

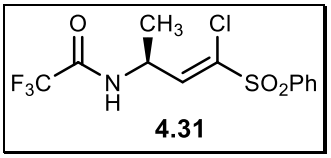

Parameter
1 Spectrometer
2 Solvent
3 Temperature
4 Spectrometer Frequency
5 Nucleus
f1 (ppm)


CH3
SO2Ph
BocHN
O

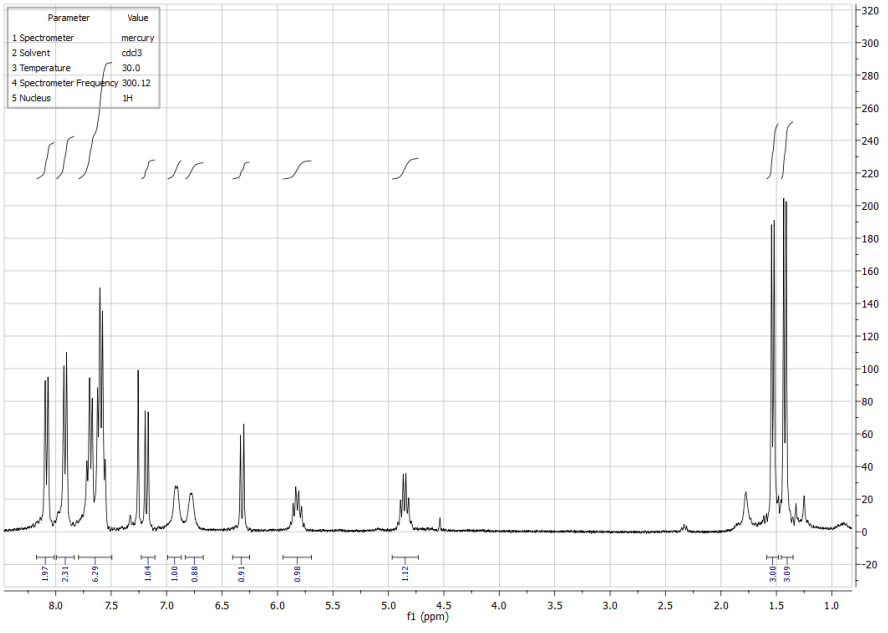
**4.30**

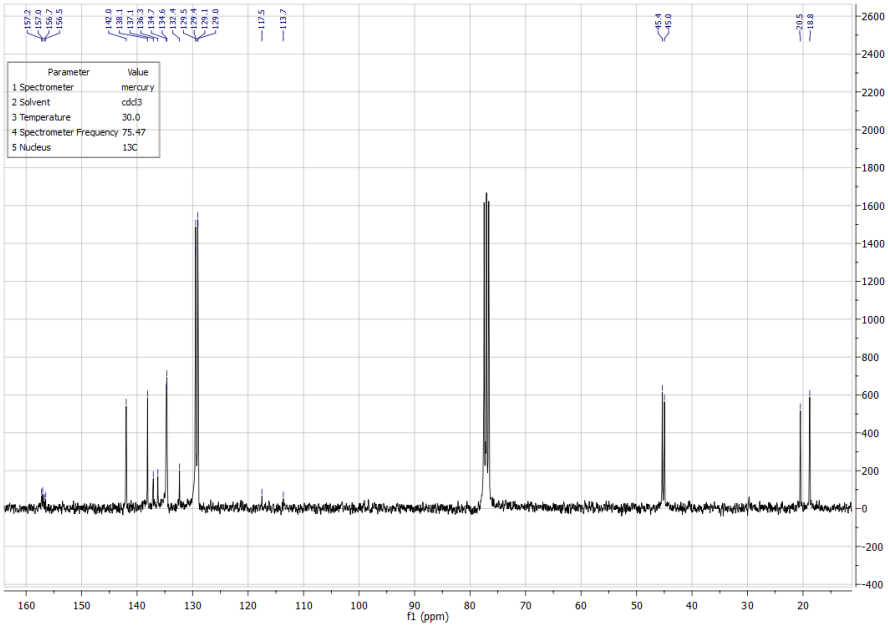

Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 499.82
5 Nucleus 1H
f1 (ppm)

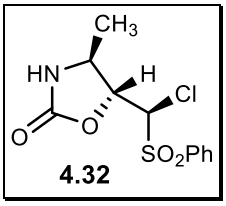

Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 125.69
5 Nucleus 13C
f1 (ppm)


O
CH3
Cl
F3C
N
H
SO2Ph

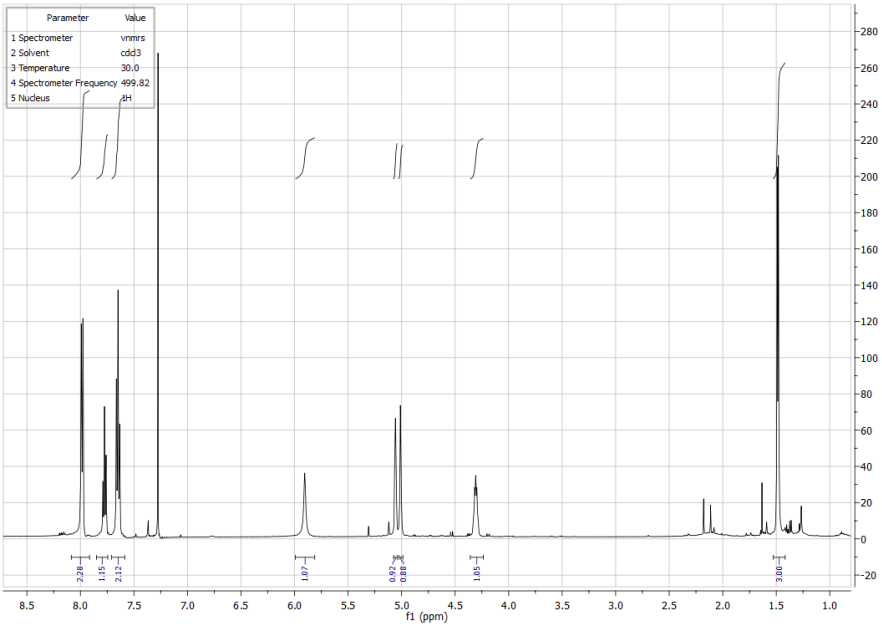
**4.31**

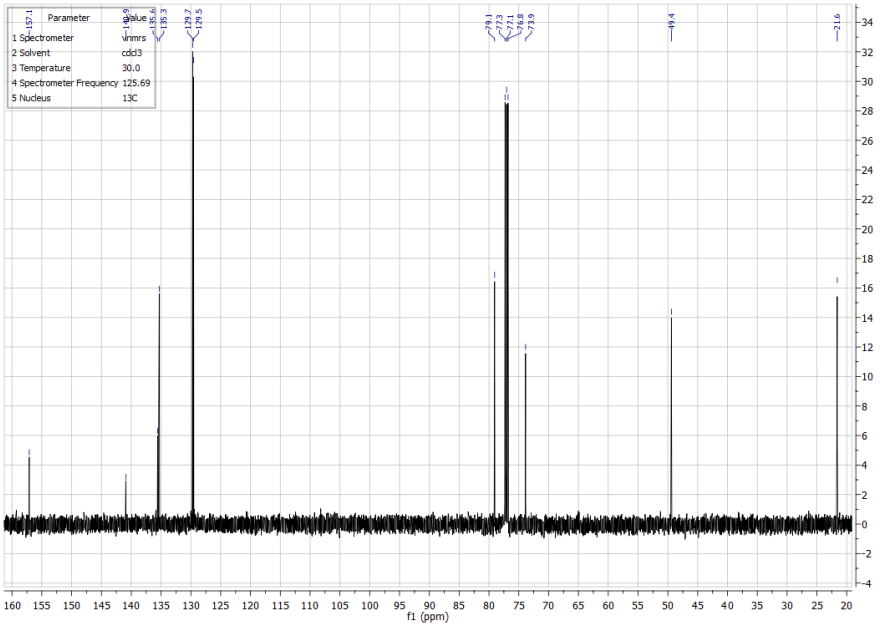

Parameter Value
1 Spectrometer mercury
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 300.12
5 Nucleus 1H
f1 (ppm)

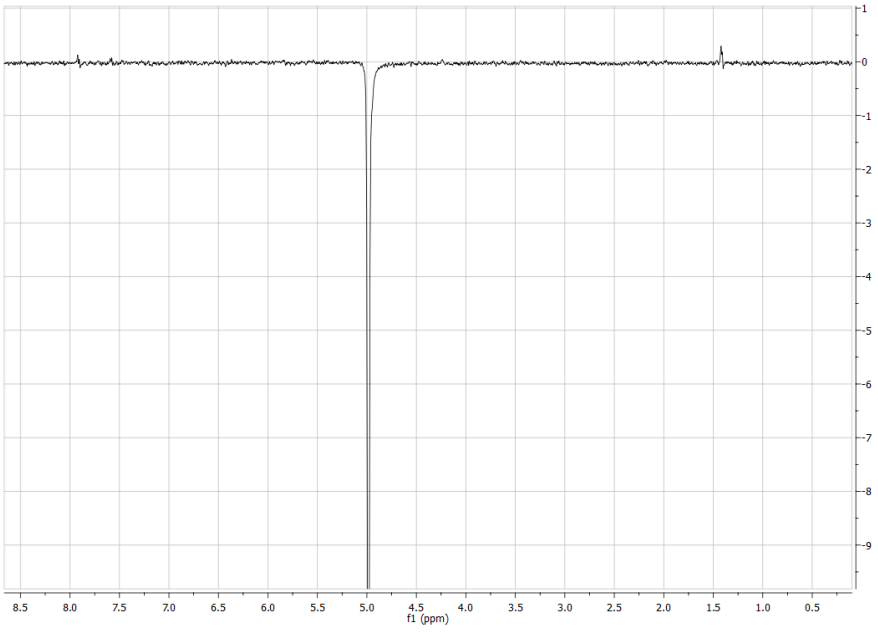

Parameter Value
1 Spectrometer mercury
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 75.47
5 Nucleus 13C
f1 (ppm)

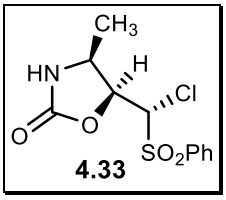

CH3
HN
H
Cl
O
O
SO2Ph

4.32

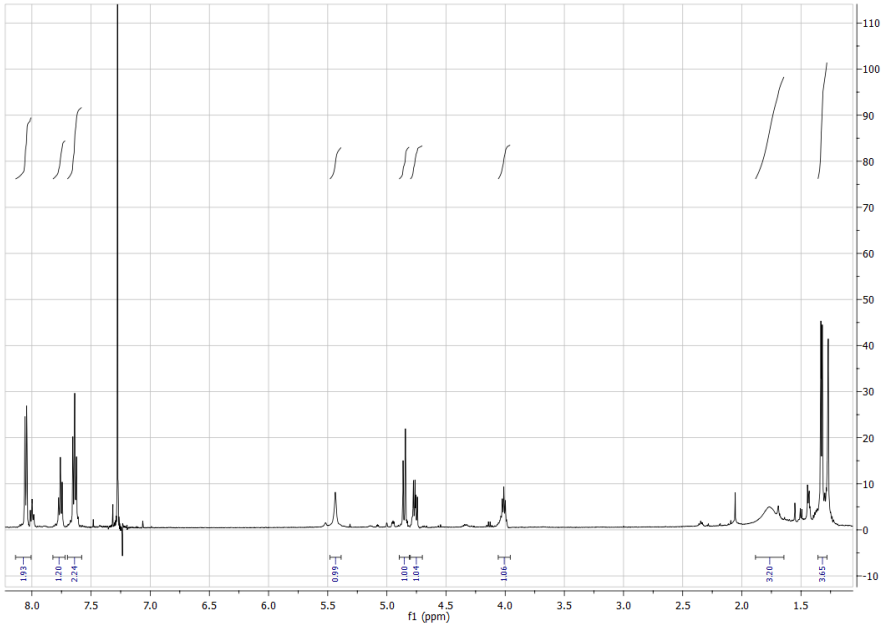

Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 499.82
5 Nucleus 1H
f1 (ppm)

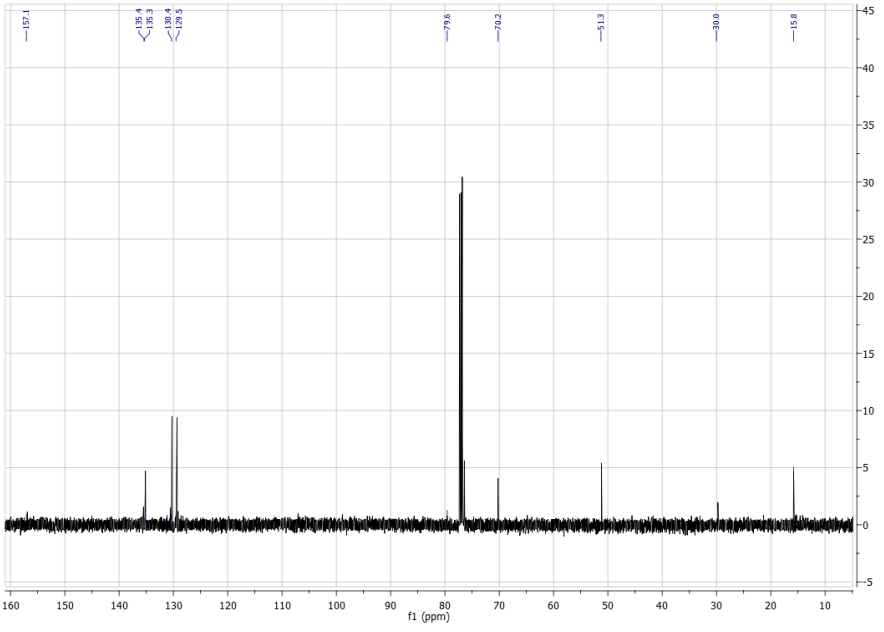

Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 125.69
5 Nucleus 13C
f1 (ppm)

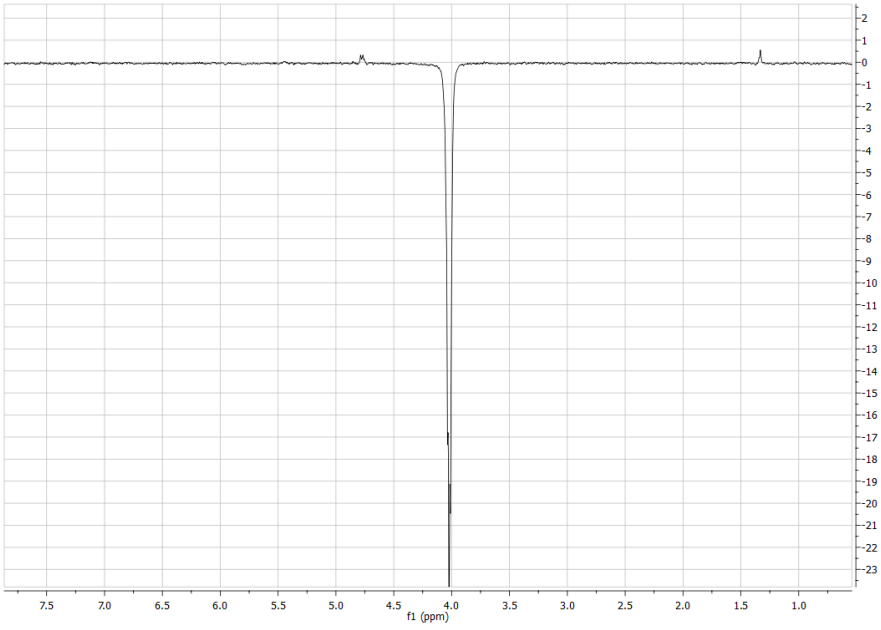

1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

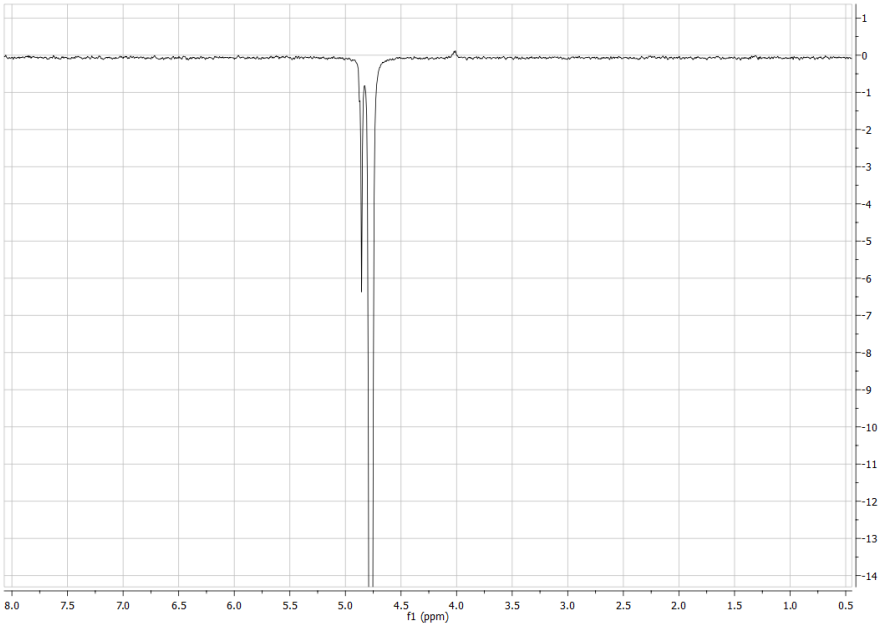

CH3
HN
H
Cl
O
O
SO2Ph

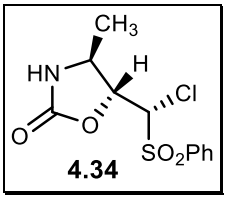
**4.33**


1.93
1.20
2.24
0.69
1.00
1.04
1.06
3.20
3.65
f1 (ppm)

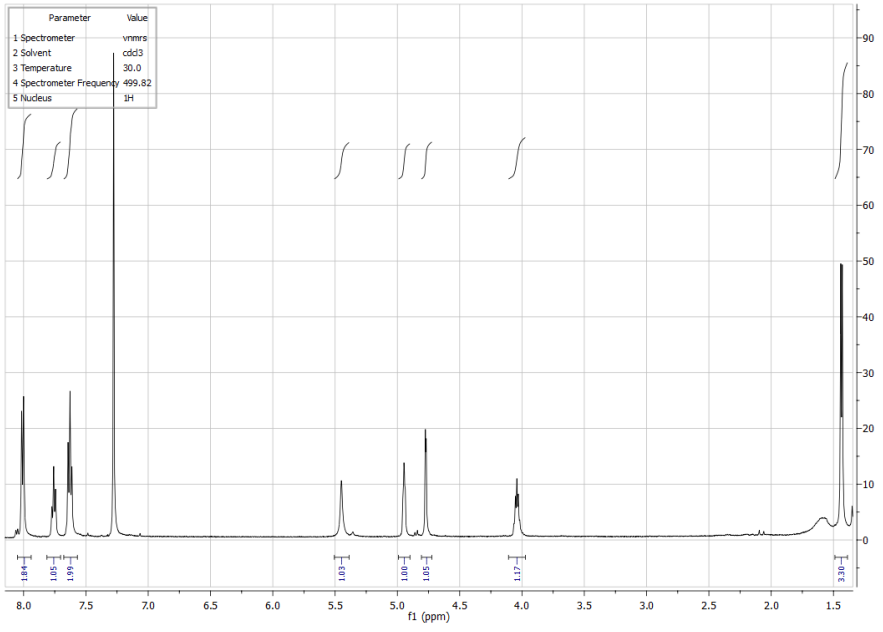

157.1
135.4
135.3
130.4
129.5
79.6
70.2
51.3
30.0
15.8
f1 (ppm)

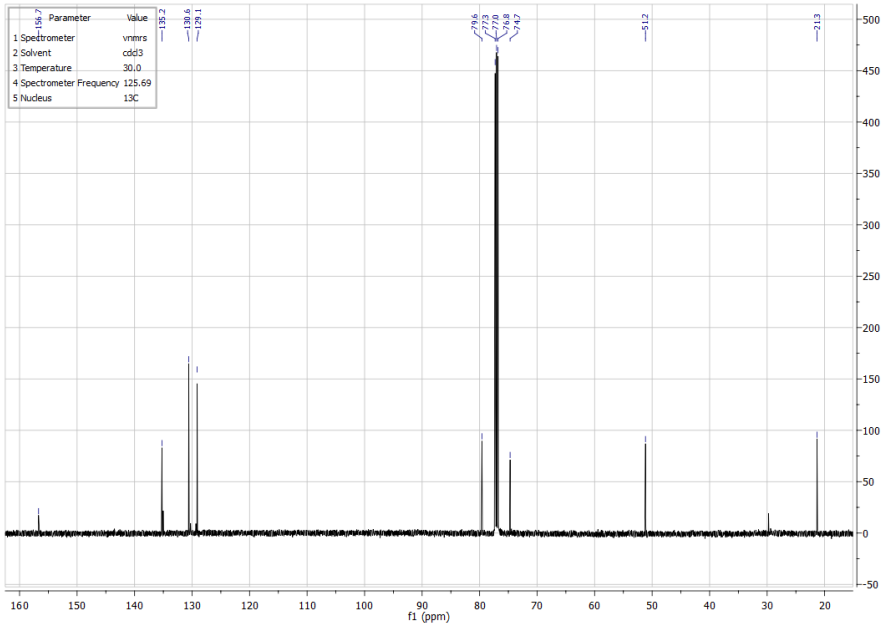

2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
-16
-17
-18
-19
-20
-21
-22
-23
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

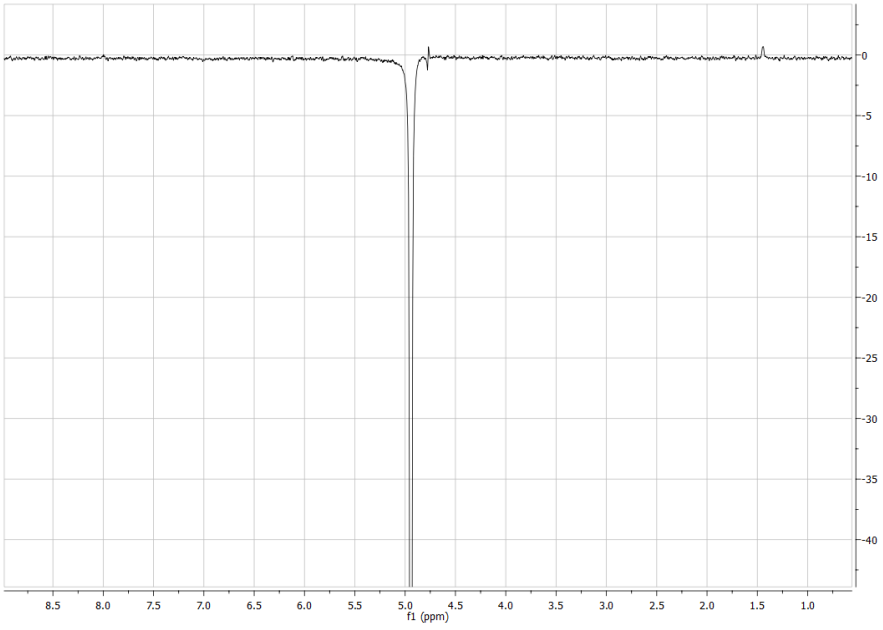

1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

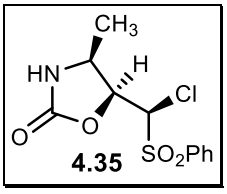

CH3
HN
H
Cl
O
O
SO2Ph

**4.34**

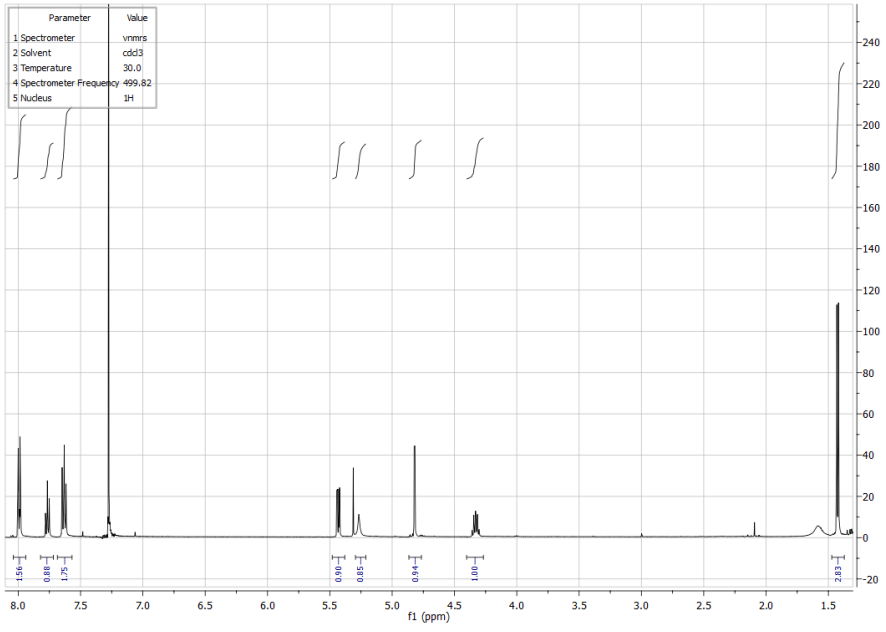

Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 499.82
5 Nucleus 1H
1.84
1.05
1.99
1.03
1.00
1.05
1.17
3.30
f1 (ppm)

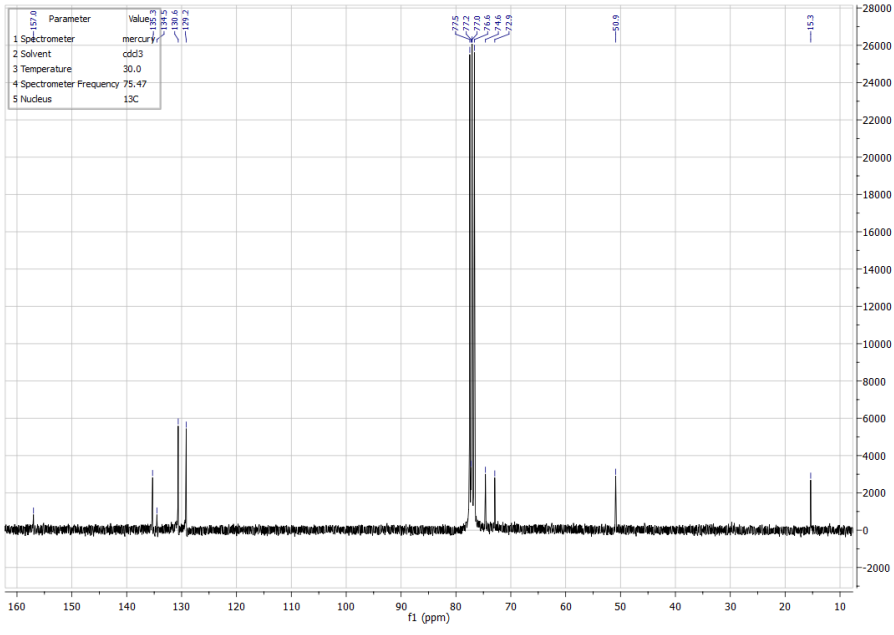

Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 125.69
5 Nucleus 13C
156.7
135.2
130.6
129.1
79.6
77.3
77.0
76.8
74.7
51.2
21.3
f1 (ppm)

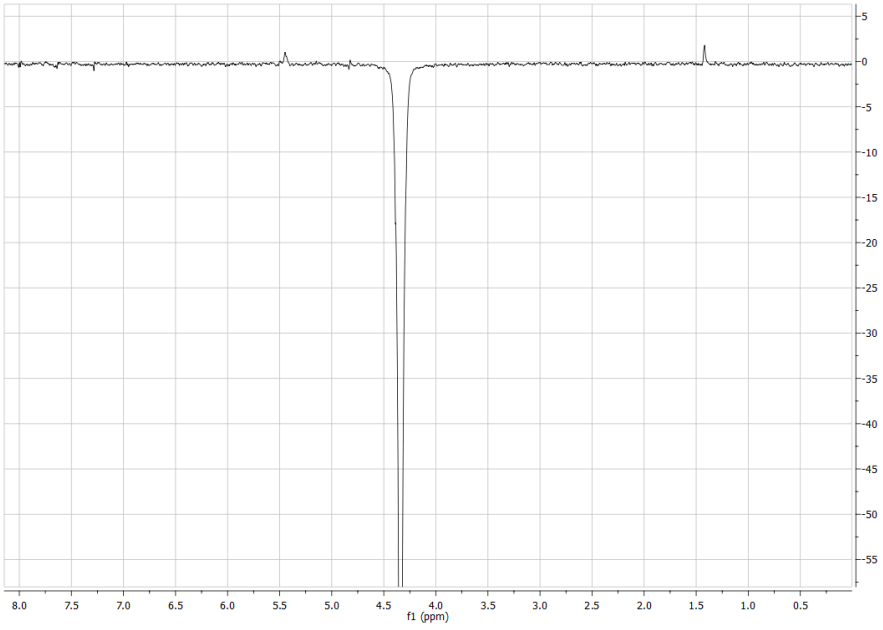

0
-5
-10
-15
-20
-25
-30
-35
-40
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

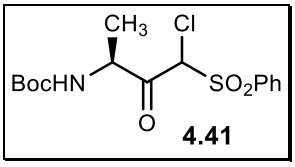

CH3
HN
H
Cl
O
O
SO2Ph

**4.35**

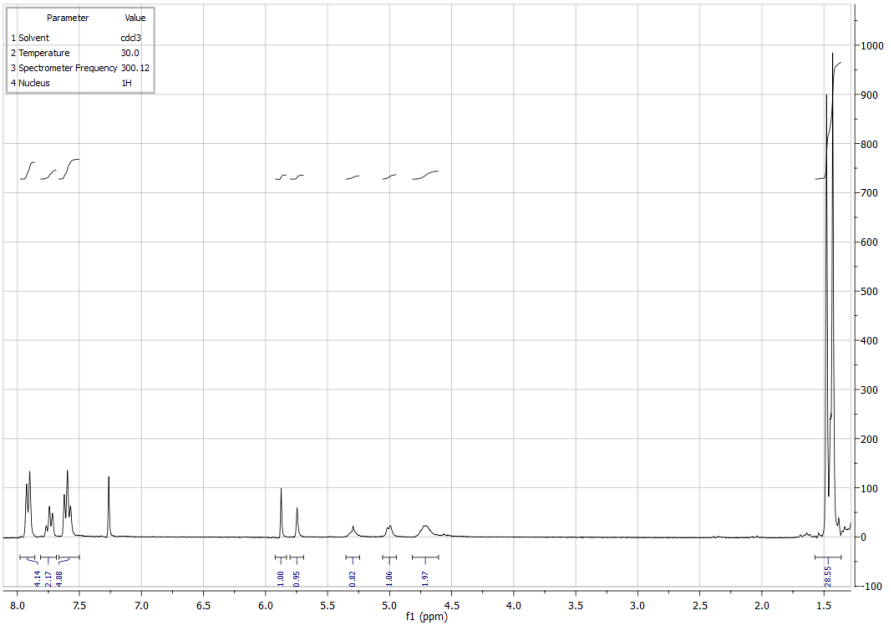

Parameter Value
1 Spectrometer vnmrs
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 499.82
5 Nucleus 1H
1.56
0.88
1.75
0.90
0.85
0.94
1.00
2.83
f1 (ppm)

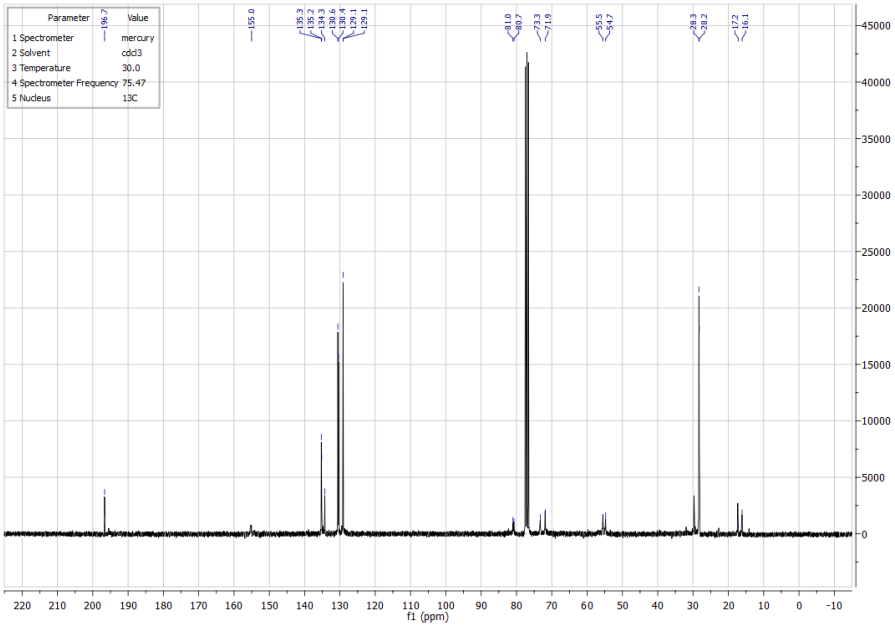

Parameter Value
1 Spectrometer mercury
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 75.47
5 Nucleus 13C
157.0
135.3
134.5
130.6
129.2
77.5
77.2
77.0
76.8
74.6
72.9
50.9
15.3
f1 (ppm)

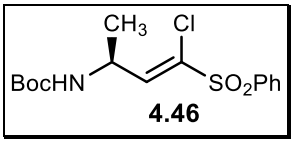

5
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
-55
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


CH3
Cl
BocHN
SO2Ph
O

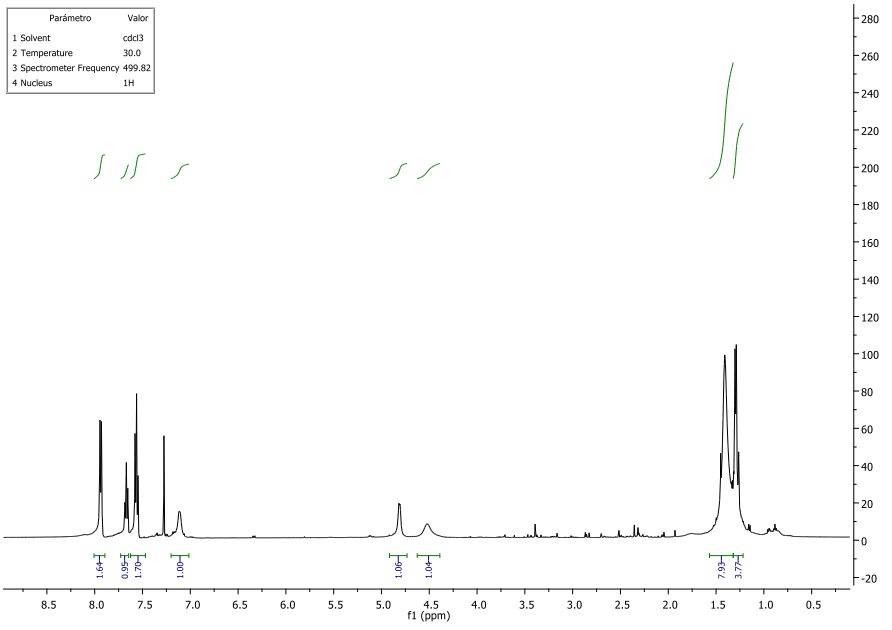
**4.41**

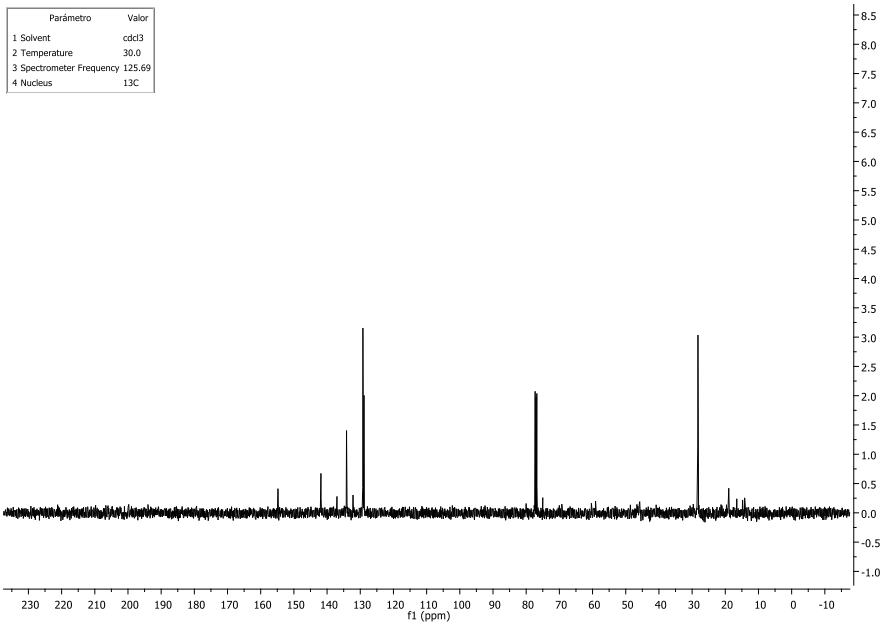

Parameter Value
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 300.12
4 Nucleus 1H
4.14
2.17
4.88
1.00
0.95
0.82
1.06
1.97
28.55
f1 (ppm)

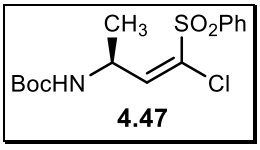

Parameter Value
1 Spectrometer mercury
2 Solvent cdcl3
3 Temperature 30.0
4 Spectrometer Frequency 75.47
5 Nucleus 13C
196.7
155.0
135.3
135.2
134.3
130.6
130.4
129.1
129.1
81.0
80.7
73.3
71.9
55.5
54.7
28.3
28.2
17.2
16.1
f1 (ppm)


CH3
Cl
BocHN
SO2Ph

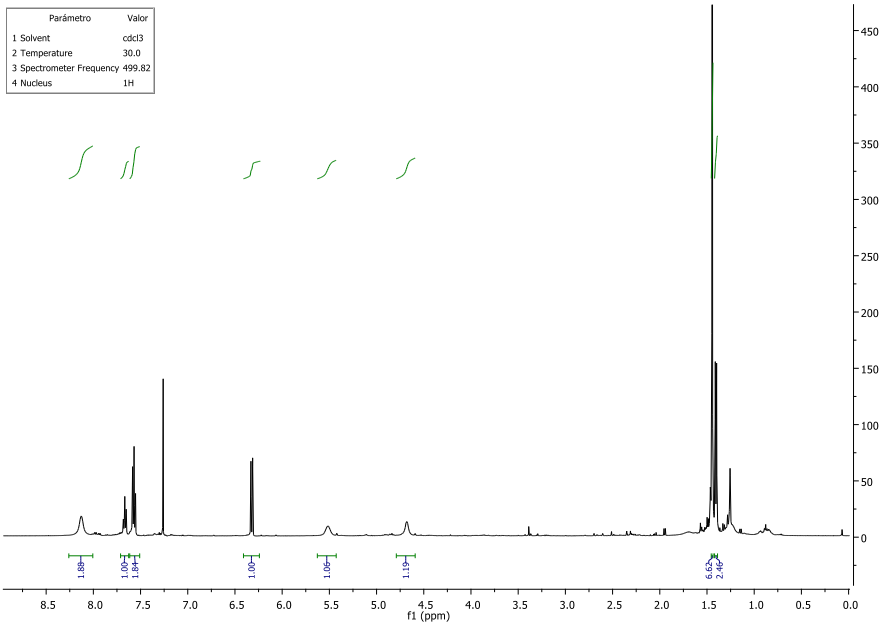
**4.46**

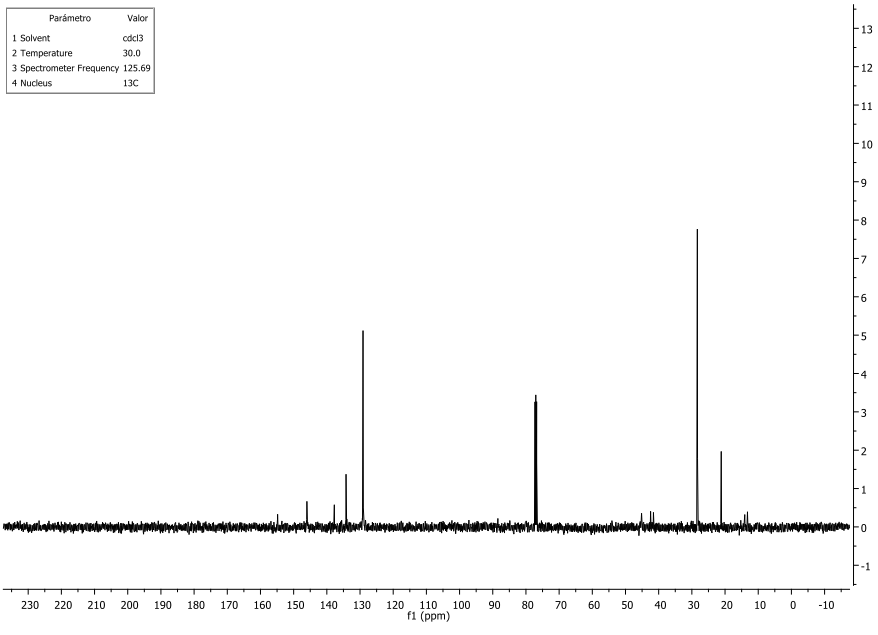

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
1.64
0.95
1.70
1.00
1.06
1.04
7.93
3.77
f1 (ppm)

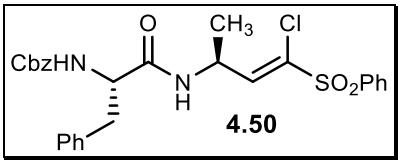

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)


CH3
SO2Ph
BocHN
Cl

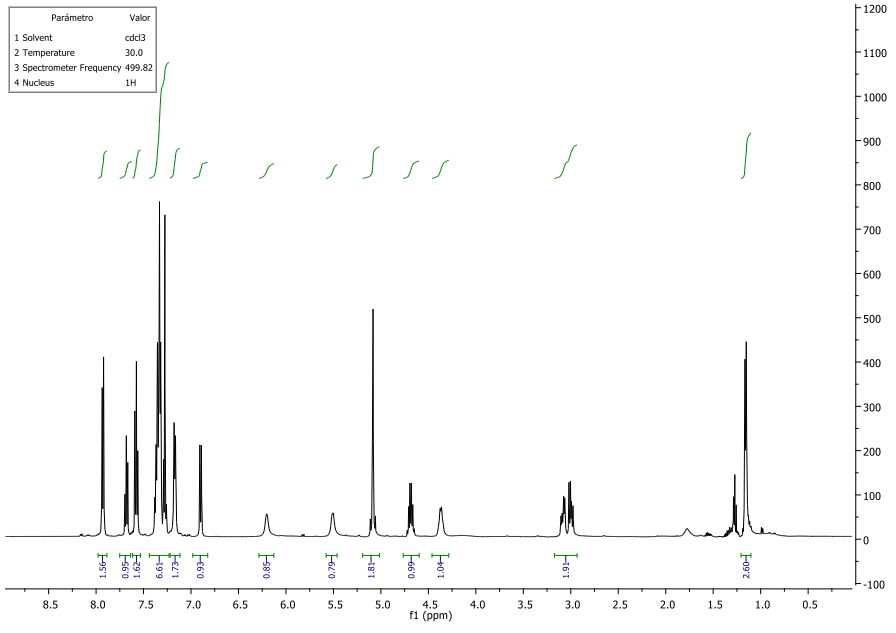
**4.47**

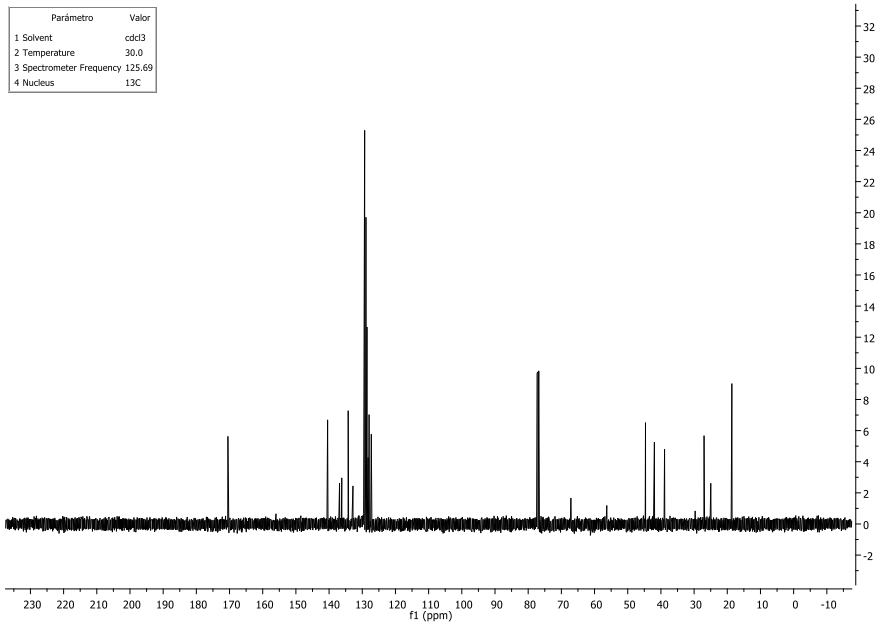

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)

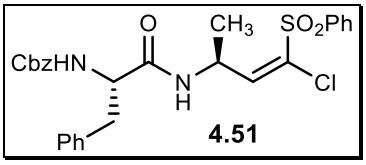

Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)


O
CH3
Cl
CbzHN
N
H
SO2Ph
Ph

**4.50**


Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 499.82
4 Nucleus 1H
f1 (ppm)


Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)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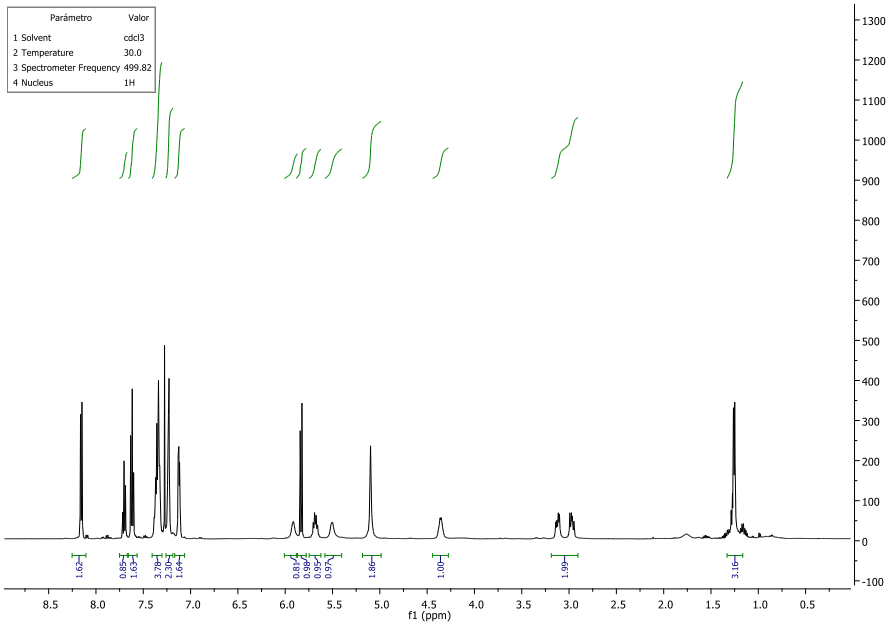
CbzHN
N
H
Cl
Ph

**4.51**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1.63
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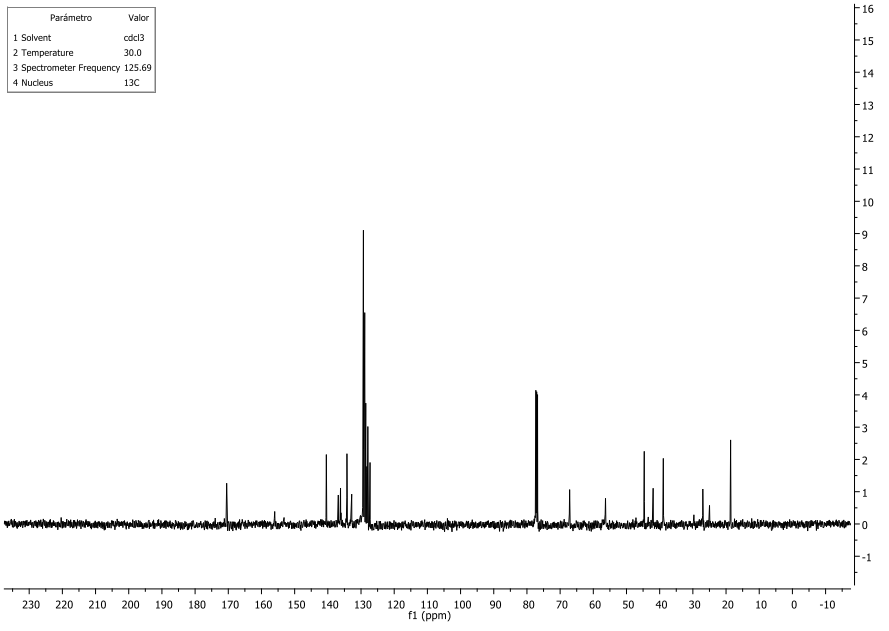
1.99
3.16
f1 (ppm)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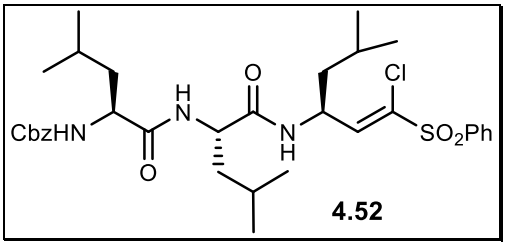
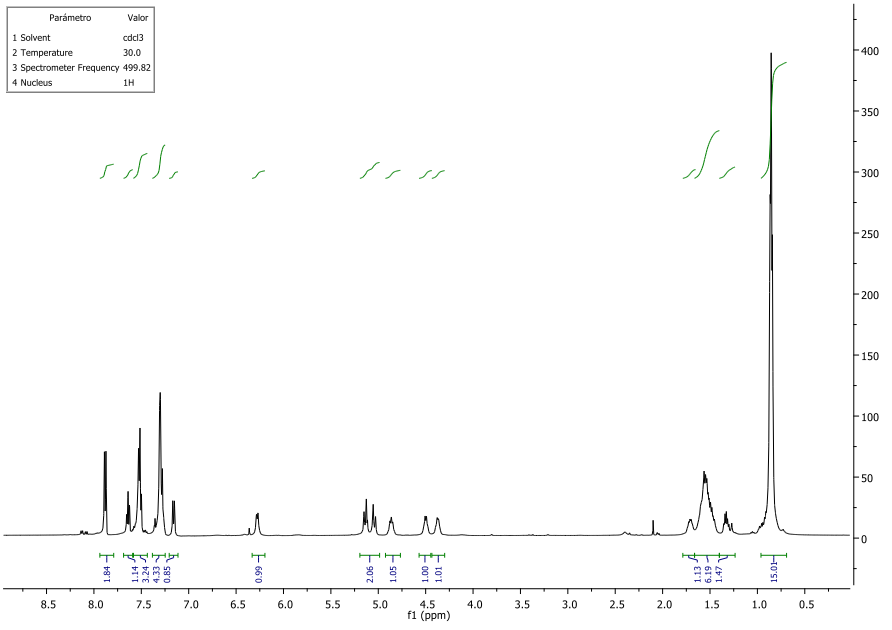
f1 (ppm)


CbzHN
O
H
N
O
N
H
Cl
SO2Ph

**4.52**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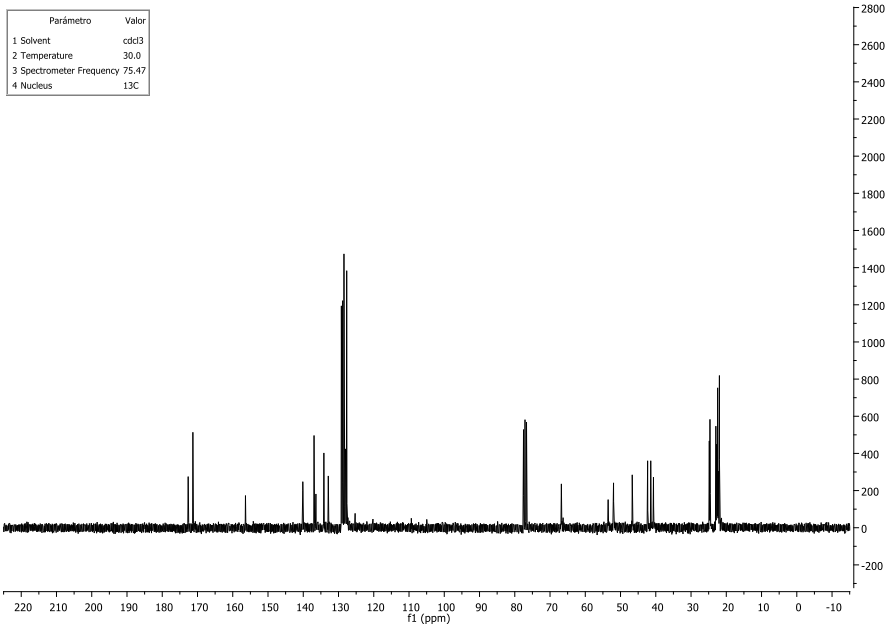
4 Nucleus 1H
f1 (ppm)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4 Nucleus 13C
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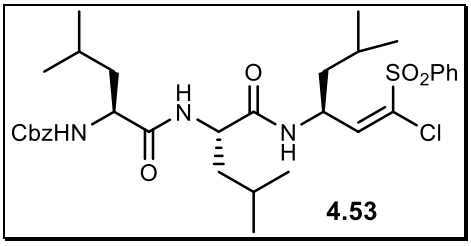
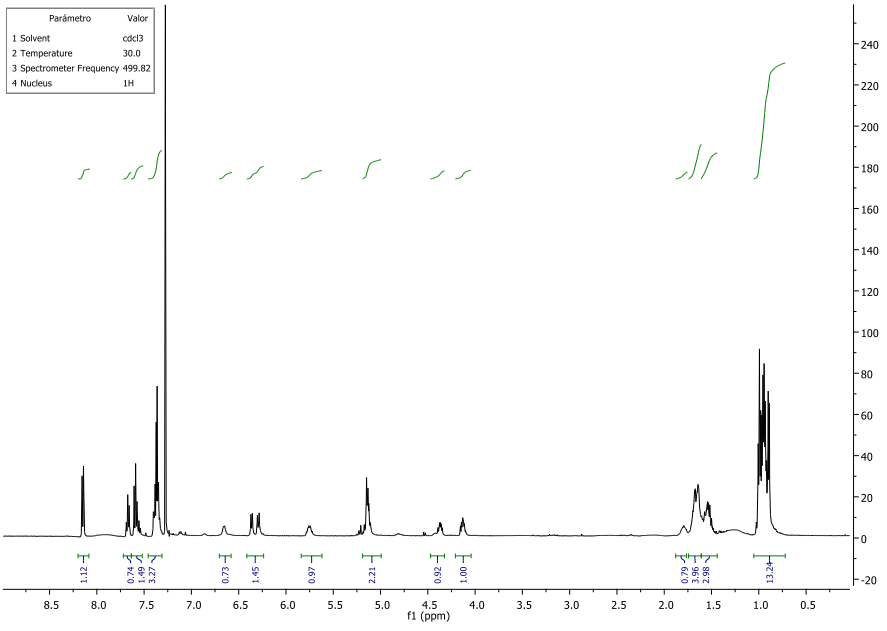

CbzHN
O
H
N
O
N
H
SO2Ph
Cl

**4.53**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3 Spectrometer Frequency 499.82
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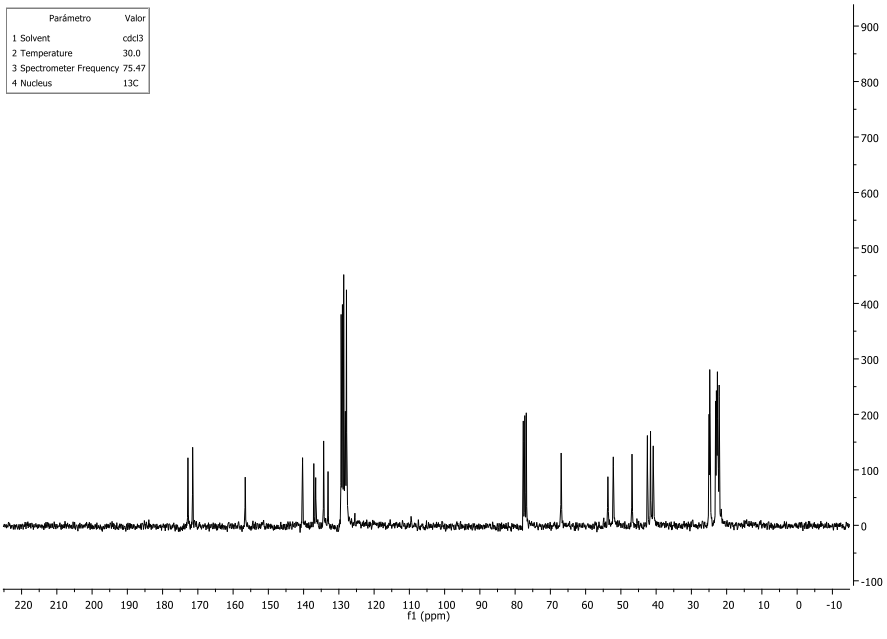
2.98
13.24
f1 (ppm)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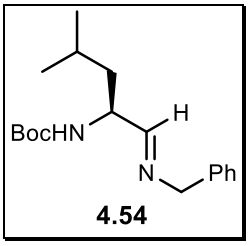
f1 (ppm)

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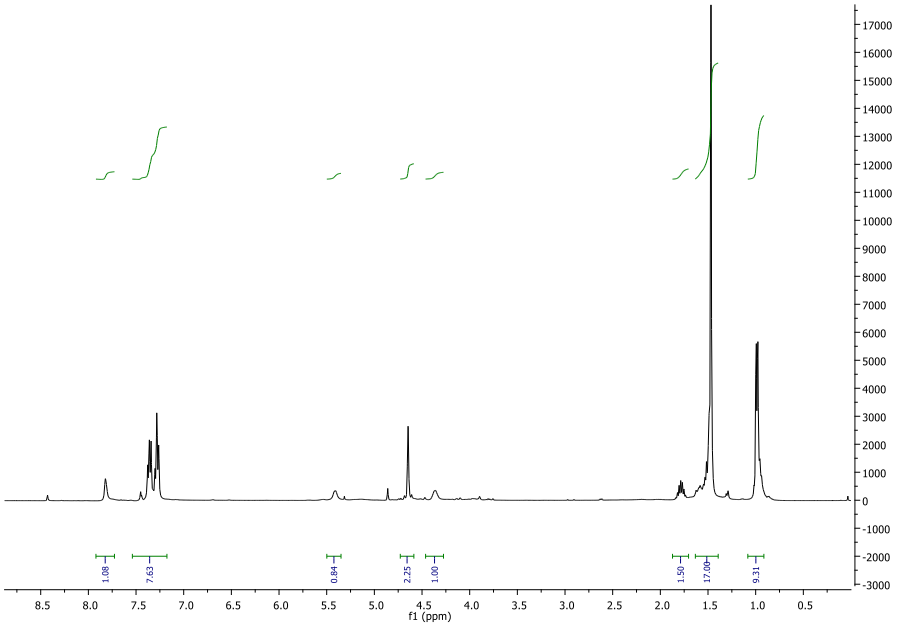


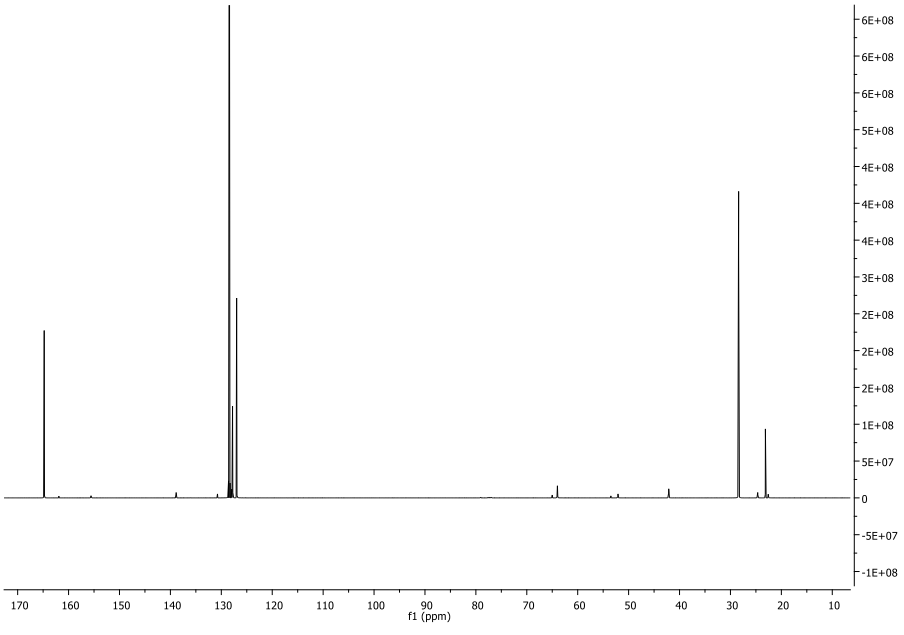

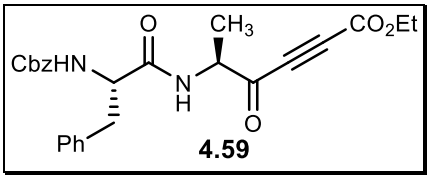
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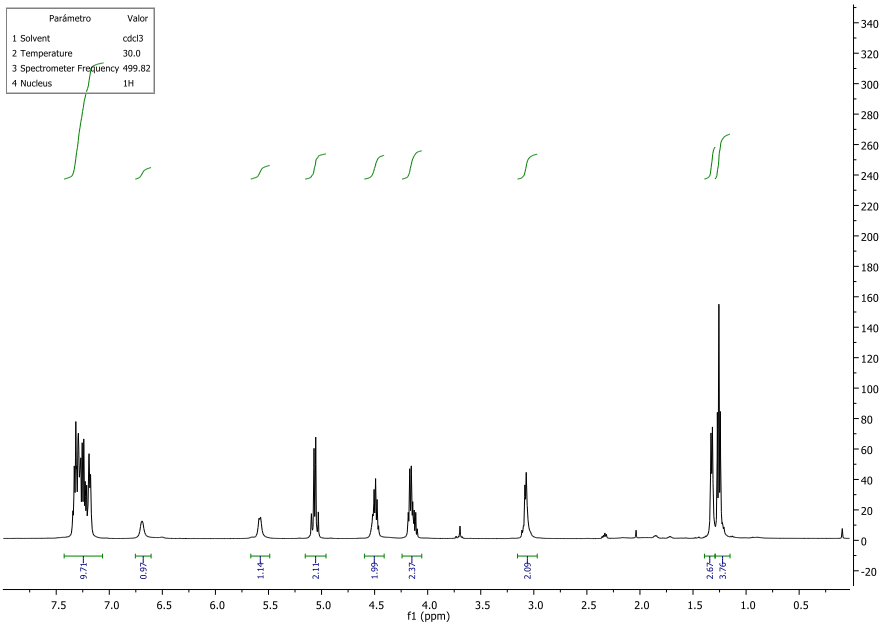
CO2Et
CbzHN
N
H
O
Ph

**4.59**


Parámetro Valor
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3 Spectrometer Frequency 499.82
4 Nucleus 1H
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2.11
1.99
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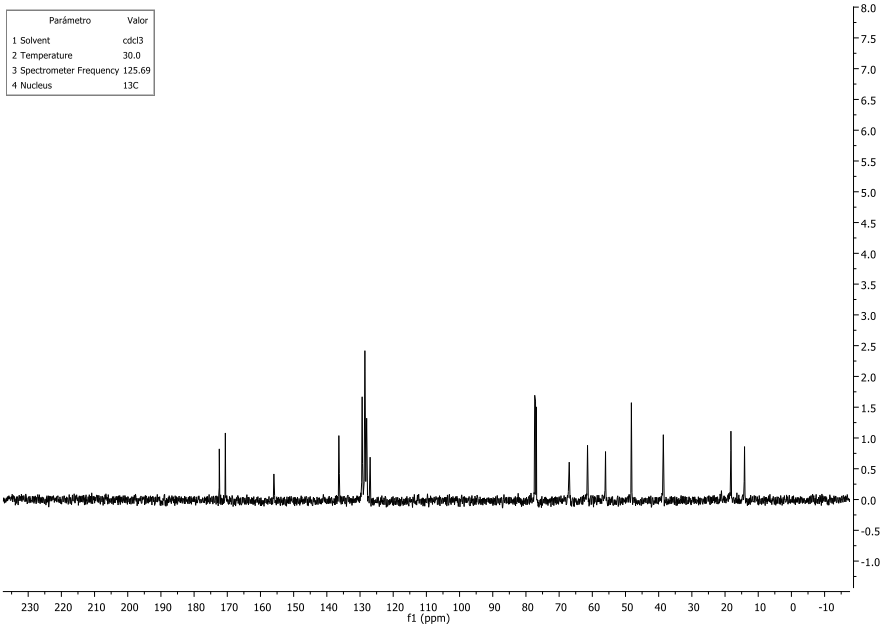
2.09
2.67
3.76
f1 (ppm)


Parámetro Valor
1 Solvent cdcl3
2 Temperature 30.0
3 Spectrometer Frequency 125.69
4 Nucleus 13C
f1 (ppm)

# Annex

# Publications

# Radical Mechanism in the Elimination of 2-Arylsulfinyl Esters

Antonio Latorre†, Irakusne López†, Victoria Ramírez†, Santiago Rodríguez†, Javier Izquierdo†,Florenci V. González*†, and Cristian Vicent*†‡

†Departament de Química Inorgànica i Orgànica and ‡Serveis Centrals d'Instrumentació Científica,Universitat Jaume I, 12080 Castelló, Spain

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DOI: 10.1021/jo300699w

Publication Date (Web): May 15, 2012



***Dedication***

We dedicate this work to the memory of Prof. Purificación Escribano.

JOC The Journal of Organic Chemistry — Note — pubs.acs.org/joc

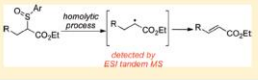
## Radical Mechanism in the Elimination of 2-Arylsulfinyl Esters

Antonio Latorre,† Irakusne López,† Victoria Ramírez,† Santiago Rodríguez,† Javier Izquierdo,† Florenci V. González,*,† and Cristian Vicent*,†,‡

†Departament de Química Inorgànica i Orgànica and ‡Serveis Centrals d'Instrumentació Científica, Universitat Jaume I, 12080 Castelló, Spain

Supporting Information

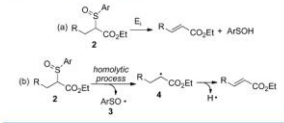
**ABSTRACT:** The mechanism of the dehydrosulfenylation of 2-arylsulfinyl esters was investigated. The reaction was found to follow a homolytic cleavage mechanism as verified by electrospray ionization tandem mass spectrometry and experimental work. Rearranged sulfoxides are obtained as byproduct during the elimination reaction.




Elimination reactions of sulfoxides and sulfones are well-known reactions in synthetic organic chemistry.[1] In particular, the transformation of 2-arylsulfinyl esters to afford unsaturated carbonyl compounds by elimination is a common synthetic transformation.[2−5] The elimination reaction of sulfoxides follows an Ei (elimination internal) mechanism, as demonstrated by the seminal work by Cram and Kingsbury,[6] who also suggested the existence of a competing homolytic mechanism for some sulfoxides having a group to stabilize a radical intermediate. Although extensive work has been reported for the elimination of sulfoxides through an Ei mechanism,[7,8] no convincing evidence has appeared for a radical mechanism involving any sulfinyl substrate.

In the context of our investigations with sulfoxides,[9,10] we became interested in determining the nature of the mechanism during the elimination reaction of 2-arylsulfinyl esters. We considered two plausible mechanistic routes for the elimination of a 2-arylsulfinyl ester to afford the corresponding enoate (Scheme 1): (a) the concerted elimination reaction (Ei) or (b) a radical process involving the homolytic scission of the C−S bond to furnish free radical 4 and aryl sulfinyl radical 3.

Scheme 1. Possible Mechanistic Routes




We undertook the study of the elimination reaction of 2-arylsulfinyl esters with a range of R alkyl groups and Ar aryl groups attached to the sulfur atom. 2-Arylsulfinyl esters 2a−f were prepared from the corresponding 2-bromo esters by treatment with the corresponding sodium thiophenolates, which furnished the expected thioethers 1a−f. Upon oxidation of the thioethers, sulfoxides 2a−f were obtained as a mixture of stereoisomers (Scheme 2).[11] Then compounds 2a−f were submitted to elimination by heating to reflux in toluene for 5 h, affording the corresponding *E*-enoates.[12] 2-Arylsulfinyl esters 2a−c gave rise to ethyl acrylate 5 and compounds 2d, 2e, and 2f afforded enoates 6, 7, and 8, respectively, as dominant products.[13]

Careful isolation of all the products obtained during the elimination reaction of compound 2a gave the phenyl-thiosulfonate 9 (that partly converts to diphenyl disulfide and sulfonic acid, see below) and rearranged sulfoxide **10** as a minor product (15%) (Scheme 3).[14]

It is also noteworthy that the unprecedented rearrangement of 2-arylsulfinyl ester 2a to yield **10** provided an alternative synthetic entry to 3-arylsulfinyl ester. To determine whether the formation of minor compound **10** was an inter- or an intramolecular process, we conducted a crossover experiment between 2b and compound 2f (see Scheme 4).

After heating of both sulfoxides 2b and 2f, the $^1$H NMR or $^{13}$C NMR spectra were highly crowded due to the presence of numerous species, making it difficult to identify the newly formed species. However, after removal of volatile acrylates and chromatographic separation of the fraction containing the 3-arylsulfinyl esters and the thiolsulfonate esters, electrospray ionization mass spectrometry (ESI−MS) analysis, operated in positive ion mode (ESI(+)), unravelled a rapid and direct snapshot of the formed species in the reaction mixture (see Figures S1 and S2, Supporting Information) and demonstrated that the rearrangement was an intermolecular rather than an intramolecular process.[15] A similar result was observed when sulfoxides 2b and 2e were heated at 80 °C for 2 h as judged by ESI−MS analysis.

The corresponding 2-arylsulfonyl esters were also prepared. When 2-arylsulfonyl esters or 2-arylthioethers (1a−f) underwent the same experimental conditions for elimination as the sulfoxides, only starting material was recovered, thus illustrating

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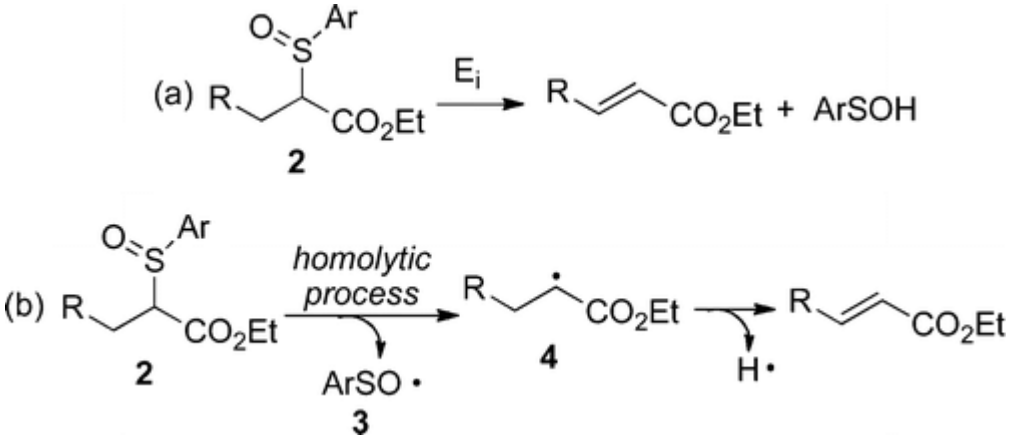
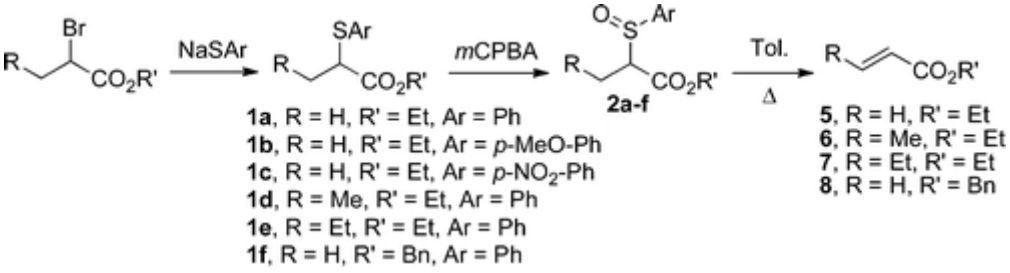
In the context of our investigations with sulfoxides,(9, 10) we became interested in determining the nature of the mechanism during the elimination reaction of 2-arylsulfinyl esters. We considered two plausible mechanistic routes for the elimination of a 2-arylsulfinyl ester to afford the corresponding enoate (Scheme 1): (a) the concerted elimination reaction (Ei) or (b) a radical process involving the homolytic scission of the C–S bond to furnish free radical **4** and aryl sulfinyl radical **3**.



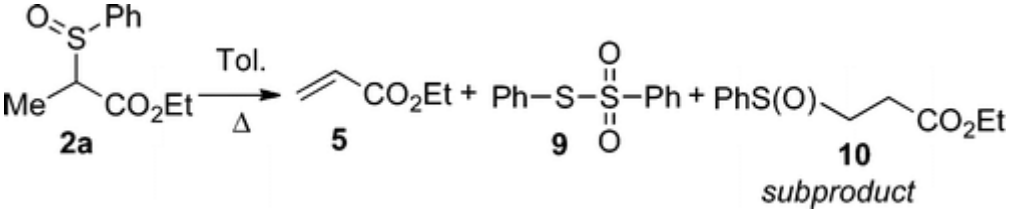

Scheme 1. Possible Mechanistic Routes

We undertook the study of the elimination reaction of 2-arylsulfinyl esters with a range of R alkyl groups and Ar aryl groups attached to the sulfur atom. 2-Arylsulfinyl esters **2a**–**f** were prepared from the corresponding 2-bromo esters by treatment with the corresponding sodium thiophenolates, which furnished the expected thioethers **1a**–**f**. Upon oxidation of the thioethers, sulfoxides **2a**–**f** were obtained as a mixture of stereoisomers (Scheme 2).(11) Then compounds**2a**–**f** were submitted to elimination by heating to reflux in toluene for 5 h, affording the corresponding *E*-enoates.(12) 2-Arylsulfinyl esters **2a**–**c** gave rise to ethyl acrylate **5** and compounds **2d**, **2e**, and **2f** afforded enoates **6**, **7**, and **8**, respectively, as dominant products.(13)



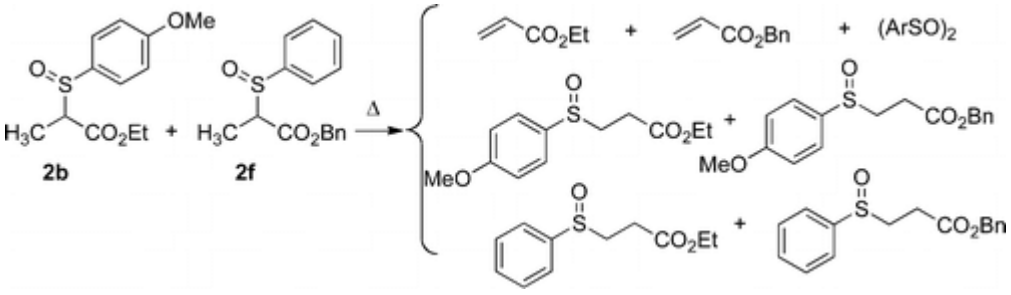

Scheme 2. Preparation and Elimination of 2-Arylsulfinyl Esters 2a–f

Careful isolation of all the products obtained during the elimination reaction of compound **2a**gave the phenylthiosulfonate **9** (that partly converts to diphenyl disulfide and sulfonic acid, see below) and rearranged sulfoxide **10** as a minor product (15%) (Scheme 3).(14)




Scheme 3. Elimination Reaction of Compound 2a

It is also noteworthy that the unprecedented rearrangement of 2-arylsulfinyl ester **2a** to yield **10**provided an alternative synthetic entry to 3-arylsulfinyl ester. To determine whether the formation of minor compound **10** was an inter- or an intramolecular process, we conducted a crossover experiment between **2b** and compound **2f** (see Scheme 4).



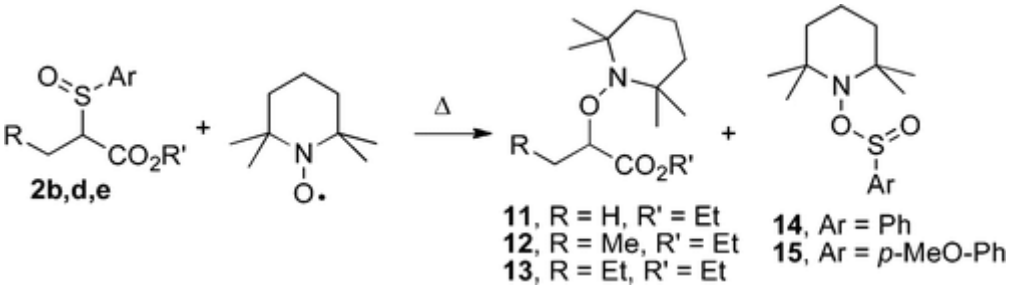

Scheme 4. Crossover Experiment between 2b and 2f

After heating of both sulfoxides **2b** and **2f**, the $^{1}H$ NMR or $^{13}C$ NMR spectra were highly crowded due to the presence of numerous species, making it difficult to identify the newly formed species. However, after removal of volatile acrylates and chromatographic separation of the fraction containing the 3-arylsulfinyl esters and the thiolsulfonate esters, electrospray ionization mass spectrometry (ESI–MS) analysis, operated in positive ion mode (ESI(+)), unravelled a rapid and direct snapshot of the formed species in the reaction mixture (see Figures S1 and S2,Supporting Information) and demonstrated that the rearrangement was an intermolecular rather than an intramolecular process.(15) A similar result was observed when sulfoxides **2b** and **2e**were heated at 80 °C for 2 h as judged by ESI–MS analysis.

The corresponding 2-arylsulfonyl esters were also prepared. When 2-arylsulfonyl esters or 2-arylthioethers (**1a**–**f**) underwent the same experimental conditions for elimination as the sulfoxides, only starting material was recovered, thus illustrating that eliminations are a characteristic feature of only the sulfoxides.

Since the photolytic homolysis of C–S bonds has been described,(16) the elimination reactions were also performed in the absence of light. When 2-arylsulfinyl esters **2a**–**f** were submitted to elimination by heating in the absence of light the products were the same as in the presence of light, thus ruling out a photolytic homolysis.

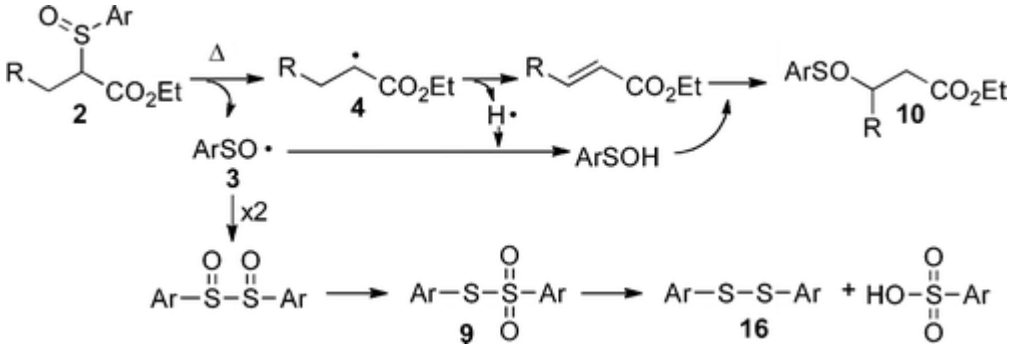
The interception of the radical intermediates would provide evidence for presence of a single-electron transfer mechanism in the elimination reaction. A nitroxyl radical (2,2,6,6-tetramethyl-1-piperidinyloxy) (Tempo) succeeded in intercepting the radical intermediates derived from 2-arylsulfinyl esters **2b**, **2d**, and **2e**. The reactions were investigated by electrospray ionization mass spectrometry (ESI–MS).(17) Compounds **2b**, **2d**, and **2e** afforded adducts **11** and **15**, **12**and **14**, and **13** and **14**, respectively. In case of the reaction using **2e** as starting material, both resulting reaction compounds **13** and **14** were isolated and fully characterized (see theSupporting Information) (Scheme 5).(18)




Scheme 5. Trapping of the Radical Intermediates Using Tempo

These results denote the occurrence of a radical process during the elimination of 2-arylsulfinyl esters into enoates. A possible mechanism is proposed in Scheme 6. Sulfoxide **2** would give rise to radical **4** and aryl sulfinyl radical **3**. Sulfinyl radicals combine to give vicinal disulfoxides (*vic*-disulfoxides) which isomerize to give phenyl thiosulfonate **9**. Thiosulfonate **9** partly converts into the corresponding diaryl sulfide **16** and sulfonic acid as it is known.(19) Radical **4** would furnish the

enoate. The formation of the subproduct **10** can be explained by addition of in situ formed phenyl sulfenic acid to the enoate. The addition of sulfenic acids to unsaturated esters have been demonstrated previously.(20-22) Compound **10** would be formed in a low yield due to the high tendency of sulfinyl radicals (or sulfenic acids) to afford thiosulphonates, although final reaction yields would depend on the subtle interplay between kinetics and thermodynamics of each elementary step depicted in Scheme 6.




Scheme 6. Radical Mechanism in the Elimination of 2-Arylsulfinyl Esters

In studies to determine the reaction mechanism of a multistep reaction, the identification of the intermediates is an essential process.(23) In this context, ESI–MS is a useful technique for analyzing chemical intermediates or products in diverse chemical and biochemical processes,(24-26) as illustrated above for the identification of the products formed in the crossover experiment. Although the detection of free radicals is sometimes difficult, ESI-–MS has been recently employed for observing radical intermediates in some processes.(27-32) For a better understanding of the mechanism of the arylsulfinyl esters elimination depicted in Scheme 6, attempts to intercept and characterize the formed species were carried out on the basis of online ESI mass spectrometry and ESI tandem mass spectrometry. Closely related thermally driven homolytic reactions have been successfully investigated by Metzgeŕs group by means of ESI mass spectrometry using modified ESI sources.(28) To investigate the elimination reaction, toluene

solutions of **2a** were heated to 100 °C and at different time intervals, aliquots were extracted, immediately diluted with hot acetonitrile, and analyzed by ESI(+) mass spectrometry. The obtained ESI mass spectra recorded at different intervals were nearly identical (Figure 1), with the most significant difference being the appearance of new signals corresponding to the $PhSSO_2Ph$ product as evidenced for the peaks assigned to $[PhSSO_2Ph + Na]^+$ ($m/z$ = 273) and $[PhSSO_2Ph + K]^+$ ($m/z$ = 289). Negative ESI mass spectrum revealed the presence of sulfonic acid as $[M - H]^-$ adduct. Diphenyl disulfide was not observed in the ESI mass spectrum because it is not readily ionized upon ESI conditions.

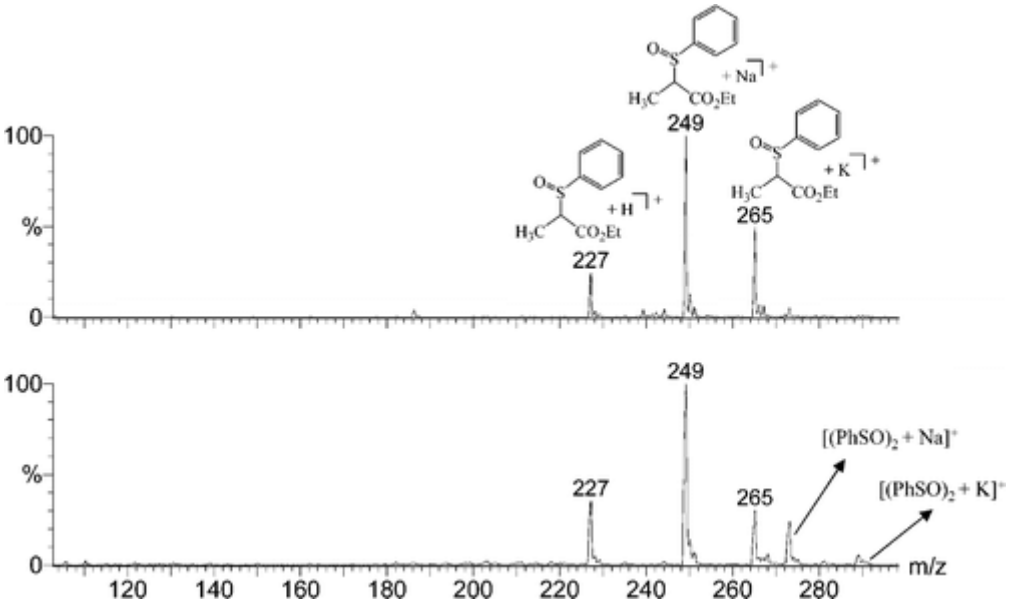



Figure 1. ESI(+) mass spectra of toluene/acetonitrile solutions of compound 2a. Samples of the initial solution of 2a (upper panel) and of compound 2a after heating for 60 min (lower panel). Note that species at m/z 227, 249, and 265 in the lower panel correspond to 2a and its rearranged isomer 10.

The transient radical cations $PhSO^{\cdot}$ and $CH_3CH{\cdot}CO_2CH_2CH_3$ (not as $H^+$, $Na^+$, or $K^+$ adducts) could not be identified in the ESI mass spectrum directly. However, monitoring the advance of the **2a** conversion to methylacrylate and the rearranged

product **10** was possible by ESI–MS, provided that both **2a** and **10** isomers at *m*/*z* = 249 displayed distinctive unimolecular dissociation upon collision-induced dissociation (CID) conditions. CID spectra of the sodium adducts of **2a** and **10** are shown in Figure 2 which allowed us to monitor the temporal evolution of the species.

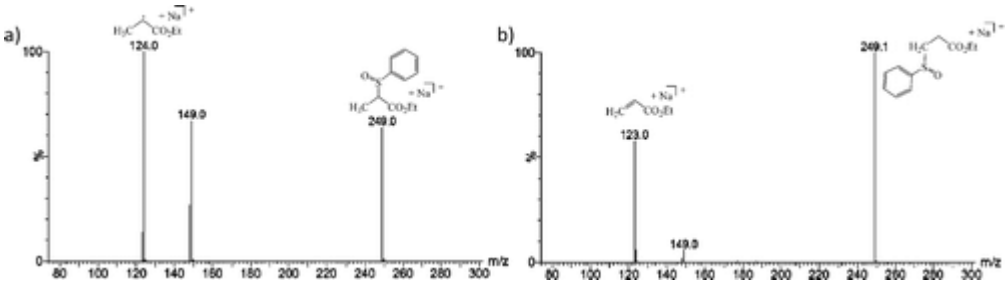



Figure 2. CID spectra of mass-selected species at (a) m/z = 249 [2a + Na]+ and (b) m/z = 249 [10 + Na]+ (Elaboratory = 15 eV).

Also helpful is the use of the MS/MS technique, where the fragment spectrum gives direct information about structural aspects of the investigated ion and can provide efficient information about mechanistic properties. It is remarkable that species [**2a** + Na]$^+$ (*m*/*z* = 249) dissociated predominantly via homolytic C–S bond cleavage affording the radical species [$CH_3CH{\cdot}CO_2CH_2CH_3$ + Na]$^+$ (*m*/*z* = 124) whereas species [**10** + Na]$^+$ (*m*/*z* = 249) dissociated via formation of the closed-shell [$CH_2CH{\cdot}CO_2CH_2CH_3$ + Na]$^+$ (*m*/*z* = 123) product ion. Moreover, at identical collision energies, it is clear that species [**2a** + Na]$^+$ is significantly easier to dissociate than [**10** + Na]$^+$ as evidenced by the relative intensity of the formed product ions. The gas-phase behavior of **2a** is reminiscent of that observed for heated solutions for which this compound is more prone to homolytically dissociate via S–C bond cleavage.

The same conditions were also used for monitoring the evolution of the reaction of 2-arylsulfinyl esters **2b**–**f**. It is remarkable that species [**2a**–**f** + Na]$^+$ unvariably

dissociated predominantly via homolytic C–S bond cleavage affording the respective radical species [$RCH_2CH{\cdot}CO_2X$ + Na]$^+$respectively, as illustrated in Figure 3 for **2b–f**. Fragmentation studies were also performed for the 2-arylsulfide esters and 2-arylsulfonyl esters but no radical intermediates were detected in their respective CID spectra. It is known that sulfides and sulfones do not eliminate as easily as sulfoxides.(33)

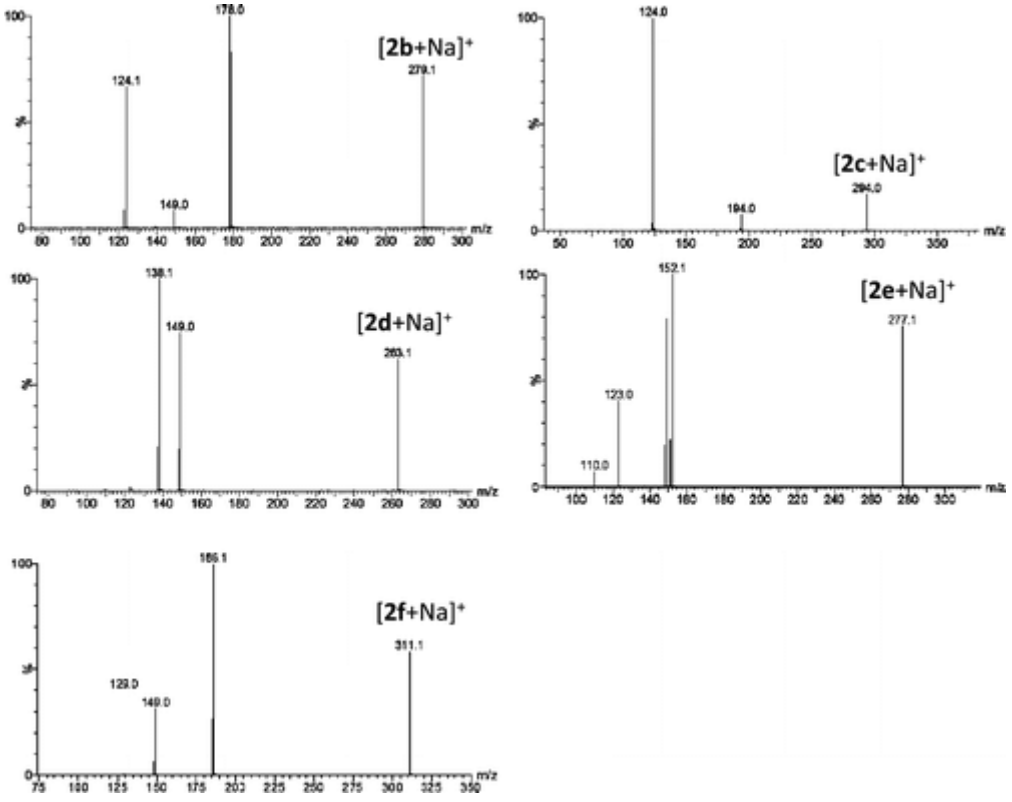
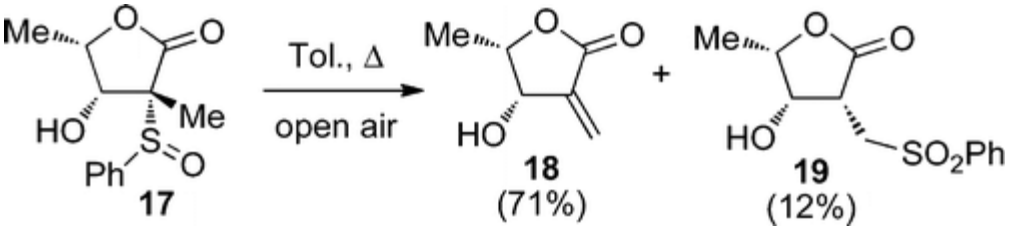



Figure 3. CID spectra of mass-selected species [2b–f + Na]+ using a collision energy (Elaboratory) = 15 eV. Radical species detected are m/z = 124 for 2b and2c, m/z = 138 for 2d, m/z = 152 for 2e and m/z = 186 for 2f.

The formation of rearrangement products during the elimination of a 2-arylsulfinyl ester was also observed during the synthesis of the natural product **18** starting from sulfoxide **17**.(9) In this case, 3-arylsulfonyl lactone **19** was obtained as a minor product along with the expected α-methylene γ-

butyrolactone **18** (Scheme 7) when the reaction was performed in open air. Sulfone**19** was formed in a highly stereoselective fashion as judged by single-crystal X-ray diffraction methods (see Figure S4, Supporting Information).(34) In this case, sulfone **19** was formed instead of the expected sulfoxide, following the well-known tendency of γ-hydroxy sulfides to be oxidized to sulfones instead of sulfoxides under photooxidation conditions.(35)




Scheme 7. Synthesis of Compounds 18 and 19

In summary, the mechanism of the dehydrosulfenylation of 2-arylsulfinyl esters for furnishing enoates has been determined to be a homolytic process. The interception of the radical intermediate using a nitroxyl radical and ESI–-MS and ESI tandem MS techniques were useful for drawing a comprehensive picture of the intermediates involved in the dehydrosulfenylation of 2-arylsulfinyl esters and suggest that a radical-mediated process is operative. An unprecedented transformation of 2-arylsulfinyl esters to 3-arylsulfinyl esters is also observed as a side reaction whose intimate mechanism is proposed.

# Stereoisomerization of α-hydroxy-β-sulfenyl-α,β-dimethyl naphthoquinones controlled by nonbonded sulfur–oxygen interactions

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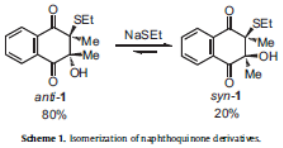
ABSTRACT

The anti α-hydroxy-β-sulfenyl-α,β-dimethyl naphthoquinones isomerize in basic media into syn/anti mixtures of isomers, giving the syn isomer as the major product. Conversely, *anti* α-hydroxy-β-alkoxy-α,β-dimethyl naphthoquinones isomerize to furnish the anti isomer as the major product. The crystal structure of *syn* α-hydroxy-β-phenylsulfenyl-α,β-dimethyl naphthoquinone has been determined. The X-ray and experimental work demonstrated that an attractive 1,4 intramolecular interaction of divalent sulfur with hydroxyl oxygen is the driving force for the aforementioned stereochemical preference.



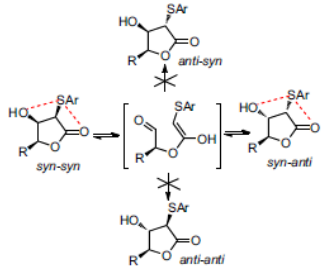
## 1. Introduction

In 1981, Silverman investigated the mechanism of vitamin K epoxide-reductase using 2,3-dimethyl-1,4-naphthoquinone 2,3-epoxide as a model for vitamin K 2,3-epoxide.[1,2] This study advanced our understanding of the mechanism of vitamin K epoxide-reductase during the catalytic conversion of vitamin K 2,3-epoxide into vitamin K, which is essential for blood coagulation. This author reported that *anti* α-hydroxy-β-ethylsulfenyl-α,β-dimethyl naphthoquinone (*anti*-**1**) isomerized into an 8:2 mixture of syn/anti isomers when treated with sodium ethylthiolate (Scheme 1) through a retro-aldol/aldol mechanism. A similar result was observed for α-hydroxy-β-phenylsulfenyl-α,β-dimethyl naphthoquinone **2**.[2]




**Scheme 1.** Isomerization of naphthoquinone derivatives.

We have recently reported that *syn–anti*-β-hydroxy-α-sulfenyl-γ-butyrolactones isomerized into *syn–syn*-β-hydroxy-α-sulfenyl-γ-butyrolactones (Scheme 2).[3] We proposed that nonbonded sulfur–oxygen interactions could control the stereoselectivity of the reaction. When we determined the crystal structures of *syn–syn* lactones, we observed that the sulfur–oxygen distances were less than the sum of the Van der Waals radii (3.3 Å), with the angle formed by the hydroxyl oxygen, sulfur, and quaternary aromatic carbon being approximately 180°. In addition, the carbonylic oxygen–sulfur was directed <40° from the perpendicular to the C–S–C. Then two concomitant, attractive 1,4 intramolecular interactions of divalent sulfur with both the carbonyl and the hydroxyl oxygens served as the driving force to establish the stereochemical preference.




**Scheme 2.** Isomerization of β-hydroxy-α-sulfenyl-γ-butyrolactones.

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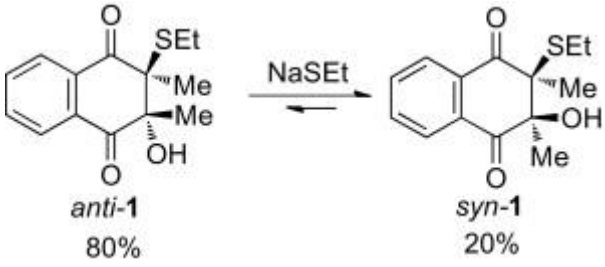
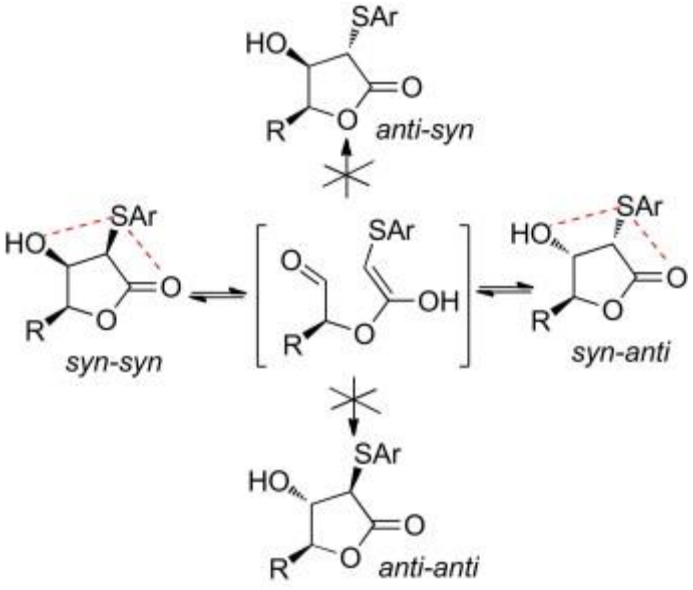
In 1981, Silverman investigated the mechanism of vitamin K epoxide-reductase using 2,3-dimethyl-1,4-naphthoquinone 2,3-epoxide as a model for vitamin K 2,3-epoxide.[1] and [2] This study advanced our understanding of the mechanism of vitamin K epoxide-reductase during the catalytic conversion of vitamin K 2,3-epoxide into vitamin K, which is essential for blood coagulation. This author reported that *anti* α-hydroxy-β-ethylsulfenyl-α,β-dimethyl naphthoquinone (*anti*-1) isomerized into an 8:2 mixture of syn/anti isomers when treated with sodium ethylthiolate ( Scheme 1) through a retro-aldol/aldol mechanism. A similar result was observed for α-hydroxy-β-phenylsulfenyl-α,β-dimethyl naphthoquinone 2. [2]




Scheme 1. Isomerization of naphthoquinone derivatives.

We have recently reported that *syn–anti*-β-hydroxy-α-sulfenyl-γ-butyrolactones isomerized into *syn–syn*-β-hydroxy-α-sulfenyl-γ-butyrolactones ( Scheme 2). [3] We proposed that nonbonded sulfur–oxygen interactions could control the stereoselectivity of the reaction. When we determined the crystal structures of *syn–syn* lactones, we observed that the sulfur–oxygen distances were less than the sum of the Van der Waals radii (3.3 Å), with the angle formed by the hydroxyl oxygen, sulfur, and quaternary aromatic carbon being approximately 180°. In addition, the carbonylic oxygen–sulfur was directed <40° from the perpendicular to the C–S–C. Then two concomitant, attractive 1,4 intramolecular interactions of divalent sulfur with both the carbonyl and the hydroxyl oxygens served as the driving force to establish the stereochemical preference.



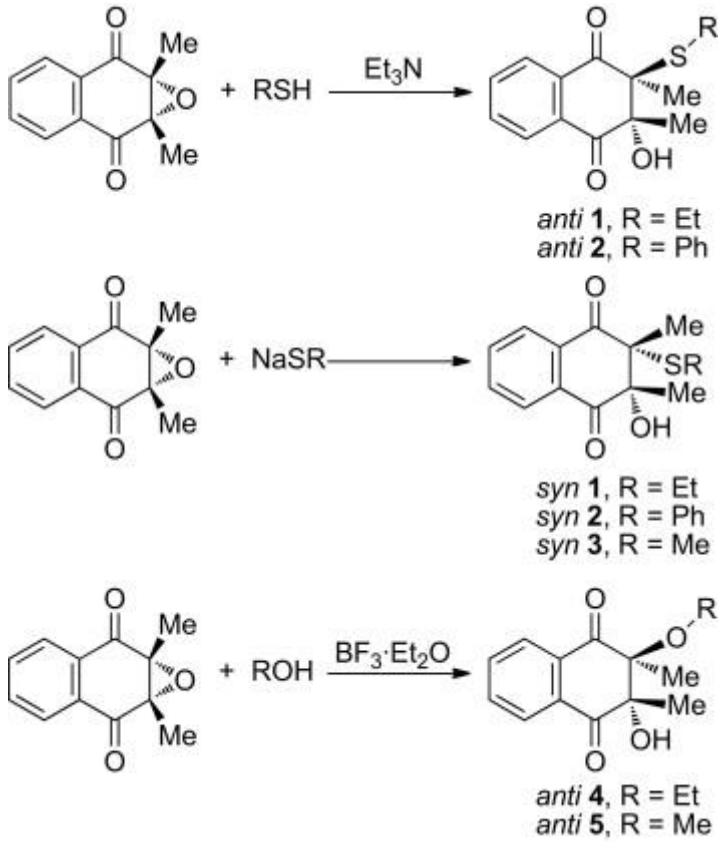

Scheme 2. Isomerization of β-hydroxy-α-sulfenyl-γ-butyrolactones.

Weak nonbonding interactions between sulfur and oxygen atoms have been invoked to explain the biological activities as well as their physical properties in a large number of organosulfur compounds.[4]

Herein, we show that for α-hydroxy-β-sulfenyl-α,β-dimethyl naphthoquinones, the intramolecular interaction of divalent sulfur with the hydroxyl oxygen also control the stereochemical preference. The crystal structure of *syn* α-hydroxy-β-sulfenyl-α,β-dimethyl naphthoquinone has been determined. Also α-hydroxy-β-alkoxy-α,β-dimethyl naphthoquinones have been prepared for comparison. The isomerization of these oxygenated analogs under the same conditions as the sulfurated ones gave either the anti isomer or an equal mixture of syn/anti isomers. α-Hydroxy-β-sulfenyl-α,β-dimethyl naphthoquinones *anti*-1 and *anti*-2 were prepared starting from the epoxide [5] using the corresponding thiol in the presence of triethylamine.[2] Having already reported compounds 1 and 2, we went on to

prepare compound *syn*3resulting from the opening of the epoxide with sodium methyl thiolate and further isomerization. [6] This reaction furnished a mixture of isomers with the syn isomer predominating.

For the preparation of the oxygenated derivatives, acidic conditions were required. The epoxide was opened with the corresponding alcohol[7] using boron trifluoride as a catalyst using conditions we had previously reported.[8] These reactions resulted to be very slow (see Experimental section). During the coagulation cascade,[9] accordingly vitamin-K-epoxide is selectively opened by a cysteine residue of vitamin-K-epoxide reductase, but it is not opened by the coagulation factors, which are serine proteases (Scheme 3).




Scheme 3. Preparation of substrates.

Compounds 1–5 were submitted to the isomerization reaction using sodium ethylthiolate or sodium phenylthiolate. Compounds 1 and 2 gave the same results as previously reported, giving rise to the syn isomer as the major form.[2] Similar results were also obtained for methyl sulfenyl derivative 3. syn-isomers 1–3 (or anti isomers 1–3 or any mixture of both) invariably isomerized into a mixture of syn/anti lactones, with the syn isomer being the major one in all cases (entries 1–9, Table 1). As already reported,2 [and] 10the elimination product was also obtained in some cases (Table 1). In contrast to β-hydroxy-α-sulfenyl-γ-butyrolactones,[3] compounds 1–3 did not isomerize in the presence of bases, such as triethylamine or *N*-methylmorpholine.

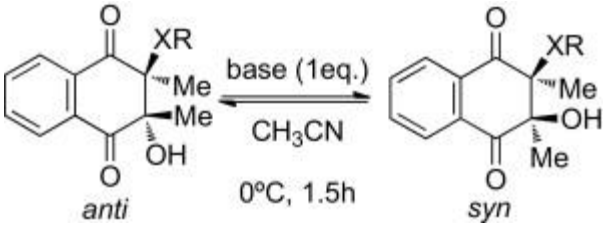
Table 1.Ratio of syn/anti isomers resulting from isomerization

| Entry | Substrate | Base | anti/syn |
|---|---|---|---|
| 1 | *syn*1 | NaSEt | 14/65[a] |
| 2 | *anti*1 | NaSEt | 14/71[a] |
| 3 | *syn*1 | NaSPh | 5/83[a] |
| 4 | *syn*2 | NaSEt | 17/83 |
| 5 | *syn*2 | NaSPh | 13/61[a] |
| 6 | *anti*2 | NaSEt | 27/68[a] |
| 7 | *anti*2 | NaSPh | 28/69[a] |
| 8 | *syn*3 | NaSEt | 8/82[a] |

| Entry | Substrate | Base | anti/syn |
|---|---|---|---|
| 9 | *syn*3 | NaSPh | 20/80 |
| 10 | *anti*4 | NaSEt | 70/30 |
| 11 | *anti*4 | NaSPh | >95/5 |
| 12 | *anti*5 | NaSEt | 58/42 |
| 13 | *anti*5 | NaSPh | 38/62 |

[a] Elimination product was already obtained.

When oxygenated substrates 4–5 were submitted to the same reaction conditions as their sulfurated counterparts, they underwent an isomerization that furnished a mixture of isomers (entries 10–13, Table 1). Compound 4 was treated with sodium ethylthiolate and sodium phenylthiolate giving rise to a mixture of isomers, with the main product being the anti isomer (entries 10 and 11). This result is opposite to the one observed starting from sulfurated compound 1, which furnished the syn isomer under the same conditions (compare 1–3 with 10–11 entries). Similarly compound 5 gave an equal mixture of syn/anti isomers whilst 3 gave the syn isomer as the main product (compare 8–9 with 12–13 entries). No elimination products were observed for compounds 4–5, as expected. Silverman had previously suggested an elimination mechanism through the formation of disulfide for compound 4.[2]




The crystal structure of compound *syn*2 has been determined ( Fig. 1). [11] The distance between the hydroxyl oxygen and sulfur was 2.97 Å. The azimuthal angle was $\varphi$=113.7° and polar angle was $\theta$=99.4° for the sulfur–hydroxyl oxygen contact. These geometric features are similar to the ones depicted for β-hydroxy-α-sulfenyl-γ-butyrolactones. [3] For them, the azimuthal angles and polar angles for sulfur–hydroxyl oxygen contacts were 107° and 93°, respectively.

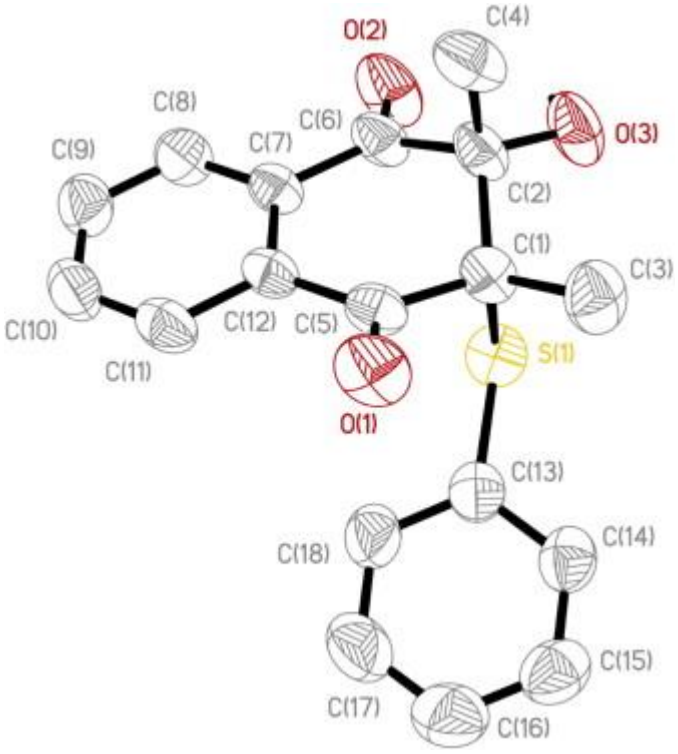



Fig. 1. X-ray structure of compound syn-2

The short atomic distance observed is interpreted as a nonbonded interaction between oxygen and sulfur atoms, an interaction that would stabilize the syn isomer.

The linear alignment of the C–S covalent bond and the coordinating hydroxyl oxygen should allows an effective orbital interaction between the oxygen lone electron pair and the σ* orbital of the S–C bond, which may elongate the S–C bond (1.77 Å for *syn*2, 1.75 Å for the diphenyl disulfide). The phenyl ring attached to the sulfur atom is oriented away from the hydroxyl, permitting the interaction to take place. Sulfur–oxygen interactions type I have nucleophilic oxygen tending to approach along the extension of the covalent bonds to sulfur. [12]

The distance between the carbonyl oxygen and sulfur was 3.55 Å. For this contact, the azimuthal angle was 94.9° and the polar angle was 97.9°. These parameters cannot be attributed to a sulfur–oxygen interaction. The planar structure of the naphthoquinone imposes rigidity that does not permit the sulfur atom to contact the carbonyl oxygen. This orientation might be at the origin of the lower selectivity observed during isomerization of compounds 1–3 as compared to the β-hydroxy-α-sulfenyl-γ-butyrolactones.

In summary, an attractive 1,4 intramolecular interaction of divalent sulfur with hydroxyl oxygen has been observed in the X-ray structure of *syn* α-hydroxy-β-phenylsulfenyl-α,β-dimethyl naphthoquinone. This sulfur–oxygen interaction can be invoked to account for the tendency of α-hydroxy-β-sulfenyl-α,β-dimethyl naphthoquinones to assume the syn configuration. This study should contribute to the understanding of the role played by this subtle noncovalent interaction in determining the biochemical processing of vitamin-K-epoxide during blood coagulation.

# Study of the stereoselectivity of the nucleophilic epoxidation of 3-hydroxy-2-methylene esters

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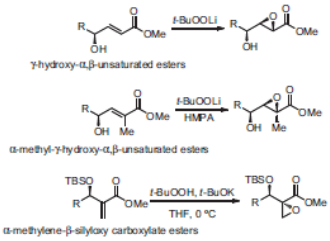
ABSTRACT

The diastereoselectivity of the nucleophilic epoxidation of 3-hydroxy-2-methylene esters has been studied. The 3-hydroxy-2-methylene esters were obtained through a Morita–Baylis–Hillman reaction. The resulting epoxyesters were treated with thiophenol for transformation into 2,3-dihydroxy-2-((phenylthio)methyl), which upon treatment with triphosgene afforded the corresponding cyclic carbonates.



## 1. Introduction

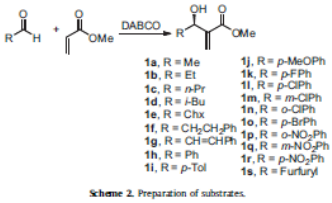
Stereoselective synthesis of α,β-epoxyesters is of considerable synthetic interest because a number of compounds can be obtained by the opening of the oxirane ring.[1–9] A convenient method for the preparation of α,β-epoxyesters is via nucleophilic epoxidation of chiral α,β-unsaturated esters.[2] We previously reported that the nucleophilic epoxidation of γ-hydroxy-α,β-unsaturated esters[8] (Scheme 1) is a diastereoselective reaction that favor the *syn* isomer. We have also reported that the stereoselectivity depends highly on the substitution of the double bond and that high *syn* stereoselectivity (dr >19:1) is observed for the α-methyl-substituted enoates[9] (Scheme 1). Free hydroxyl group resulted to be key for the control of the stereoselectivity. The nucleophilic epoxidation of methyl 2-methylene-3-*tert*-butyldimethylsilyloxycarboxylate esters has been recently reported by A. Myers to get the *anti* diastereomer with high selectivity[12] (Scheme 1). The epoxidation of Morita–Baylis–Hillman adducts is an interesting transformation because the resulting epoxides can be used in the total synthesis of interesting natural products.[10–12] We now report a study of the stereoselectivity of the nucleophilic epoxidation of β-hydroxy-α-methylene esters.




**Scheme 1.** Stereoselective nucleophilic epoxidations of unsaturated esters.

## 2. Results and discussion

We wanted to study the selectivity of epoxidation of 3-hydroxy-methylene carboxylate esters with a range of R alkyl and aryl groups (Scheme 2). For the preparation of the substrates, a comparison of different experimental procedures was performed as




**Scheme 2.** Preparation of substrates.

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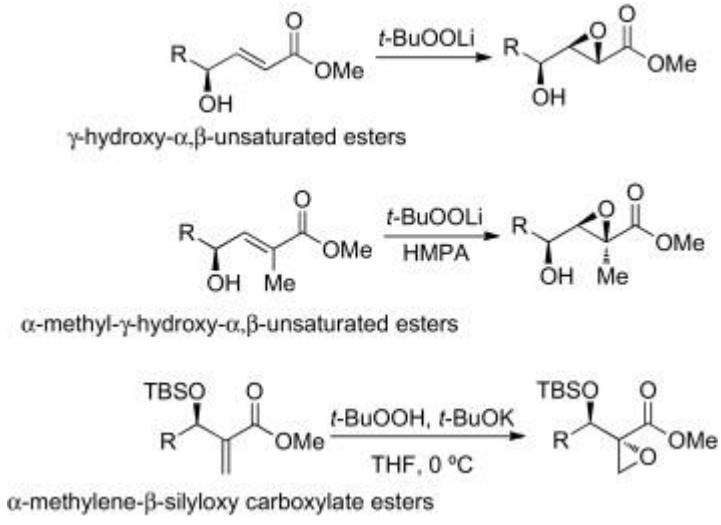
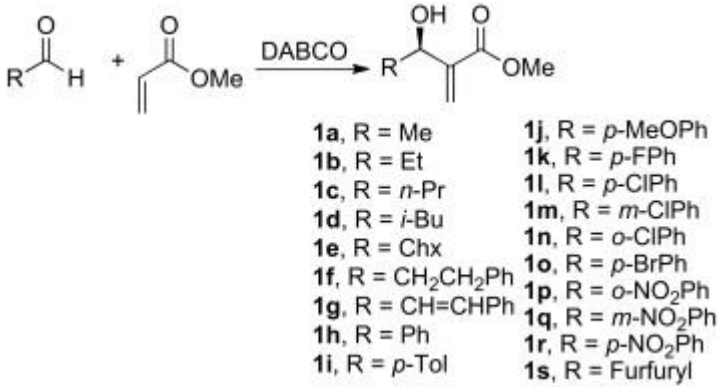
Stereoselective synthesis of α,β-epoxyesters is of considerable synthetic interest because a number of compounds can be obtained by the opening of the oxirane ring.[1, 2, 3, 4, 5, 6, 7, 8 and 9] A convenient method for the preparation of α,β-epoxyesters is via nucleophilic epoxidation of chiral α,β-unsaturated esters.[2] We previously reported that the nucleophilic epoxidation of γ-hydroxy-α,β-unsaturated esters[8] (Scheme 1) is a diastereoselective reaction that favor the *syn* isomer. We have also reported that the stereoselectivity depends highly on the substitution of the double bond and that high *syn*stereoselectivity (dr >19:1) is observed for the α-methyl-substituted enoates [9] ( Scheme 1). Free hydroxyl group resulted to be key for the control of the stereoselectivity. The nucleophilic epoxidation of methyl 2-methylene-3-*tert*-butyldimethylsilyloxycarboxylate esters has been recently reported by A. Myers to get the *anti* diastereomer with high selectivity [12] ( Scheme 1). The epoxidation of Morita–Baylis–Hillman adducts is an interesting transformation because the resulting epoxides can be used in the total synthesis of interesting natural products. [10, 11 and 12] We now report a study of the stereoselectivity of the nucleophilic epoxidation of β-hydroxy-α-methylene esters.




Scheme 1. Stereoselective nucleophilic epoxidations of unsaturated esters.

We wanted to study the selectivity of epoxidation of 3-hydroxy-methylene carboxylate esters with a range of R alkyl and aryl groups (Scheme 2). For the preparation of the substrates, a comparison of different experimental procedures was performed as shown in Table 1. Most of the substrates were prepared in good yield using DABCO as a base and a (1:1) mixture of dioxane/water as reported.[13] We obtained higher yields when the reaction was performed at higher concentrations (10 M) than reported (see Experimental section). Compounds **1i** and **j** were obtained in best yields under solvent-free conditions and longer period of time, and compounds **1m** and **n** were prepared using dimethylsulfoxide as a solvent.




Scheme 2. Preparation of substrates.

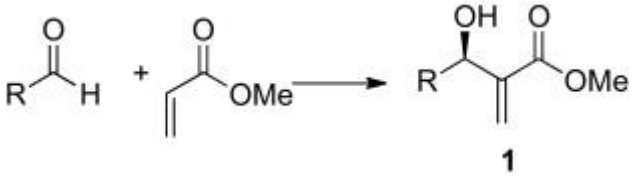



Table 1.Preparation of esters **1**

| Entry | Substrate | Conditions | Yield |
|---|---|---|---|
| 1 | **1a** | DABCO, dioxane/$H_2O$ (1:1), 10 M, 48 h, rt | 99 |
| 2 | **1b** | DABCO, dioxane/$H_2O$ (1:1), 10 M, 48 h, rt | 70 |

| Entry | Substrate | Conditions | Yield |
|---|---|---|---|
| 3 | **1c** | DABCO, dioxane/$H_2O$ (1:1), 10 M, 48 h, rt | 99 |
| 4 | **1d** | DABCO, dioxane/$H_2O$ (1:1), 10 M, 48 h, rt | 85 |
| 5 | **1e** | DABCO, dioxane/$H_2O$ (1:1), 10 M, 48 h, rt | 81 |
| 6 | **1f** | DABCO, dioxane/$H_2O$ (1:1), 10 M, 48 h, rt | 99 |
| 7 | **1g** | DABCO, dioxane/$H_2O$ (1:1), 10 M, 48 h, rt | 99 |
| 8 | **1h** | DABCO, dioxane/$H_2O$ (1:1), 12 M, 48 h, rt | 99 |
| 9 | **1i** | DABCO, solvent-free 4 days, rt | 82 |
| 10 | **1j** | DABCO, solvent-free 5 weeks, rt | 77 |
| 11 | **1k** | DABCO, dioxane/$H_2O$ (1:1), 10 M, 36 h, rt | 99 |
| 12 | **1l** | DABCO, solvent-free 5 days, rt | 94 |
| 13 | **1m** | DABCO, DMSO 7 M, 4 days, rt | 99 |
| 14 | **1n** | DABCO, DMSO 7 M, 4 days, rt | 99 |
| 15 | **1o** | DABCO, dioxane/$H_2O$ (1:1), 10 M, 36 h, rt | 89 |
| 16 | **1p** | DABCO, dioxane/$H_2O$ (1:1), 10 M, 16 h, rt | 95 |
| 17 | **1q** | DABCO, dioxane/$H_2O$ (1:1), 10 M, 16 h, rt | 87 |
| 18 | **1r** | DABCO, dioxane/$H_2O$ (1:1), 10 M, 3 h, rt | 83 |
| 19 | **1s** | DABCO, dioxane/$H_2O$ (1:1), 10 M, 20 h, rt | 85 |

Esters **1** were epoxidized using lithium *tert*-butylperoxide (2 equiv) as the oxidizing reagent in THF as solvent at –20 °C. 2, 8 and 9 Table 2 shows that the **2***syn* isomer was the major product in all cases. For the aliphatic series (compounds **1a**–**f**), the higher steric volume of the R pendant alkyl group the higher stereoselectivity is observed (entries 1–6). When the R is an alkenyl group

then the epoxidation reaction is not stereoselective (entry 7). Compounds **1h–s** having an aromatic group gave the corresponding *syn*isomer **2** in very good selectivity.

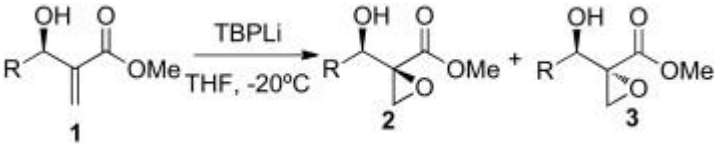



Table 2.Epoxidation of compounds **1**

| Entry | Substrate | 2/3 | Yield[a] |
|---|---|---|---|
| 1 | Me | 67/33 | 72 |
| 2 | Et | 76/24 | 70 |
| 3 | *n*-Pr | 81/19 | 79 |
| 4 | *i*-Bu | 81/19 | 71 |
| 5 | Chx | 92/8 | 85 |
| 6 | $PhCH_2CH_2$ | 77/23 | 59 |
| 7 | PhCH═CH | 53/47 | 47 |
| 8 | Ph | 93/7 | 68 |
| 9 | *p*-Tol | 89/11 | 82 |
| 10 | *p*-MeOPh | 92/8 | 73 |
| 11 | *p*-FPh | 90/10 | 65 |

| Entry | Substrate | 2/3 | Yield[a] |
|---|---|---|---|
| 12 | *p*-ClPh | 84/16 | 52 |
| 13 | *m*-ClPh | 92/8 | 38 |
| 14 | *o*-ClPh | 92/8 | 52 |
| 15 | *p*-BrPh | 90/10 | 68 |
| 16 | *o*-NO2Ph | 83/17 | 43 |
| 17 | *m*-NO2Ph | 80/20 | 60 |
| 18 | *p*-NO2Ph | 91/9 | 65 |
| 19 | Furfuryl | 93/7 | 69 |

[a] Isolated yield of products corresponds to mixtures of *syn* and *anti* diastereomers.

We also epoxidized compound **1h** by using oxidants other than lithium *tert*-butylperoxide (Table 3). If the reaction was carried out using *tert*-butyl hydrogenperoxide in the presence of substoichiometric amount of base (entry 1), then a slightly lower selectivity was observed compared to the reaction carried out using a stoichiometric amount of oxidant (entry 8, Table 2). Lithium cumylperoxide gave similar result to lithium *tert*-butylperoxide (entry 2). On the other hand, in the alkaline peroxides series, potassium gave poorer stereoselectivity than either lithium or sodium (entries 1 and 6–8). The yield of the epoxidation using *m*-CPBA (entry 3) was low at rt but it increased at higher temperature (entry 4), affording the *syn* isomer as the major one. When *m*-CPBA was used in the presence of potassium carbonate [14] (entry 5), only starting material was recovered.

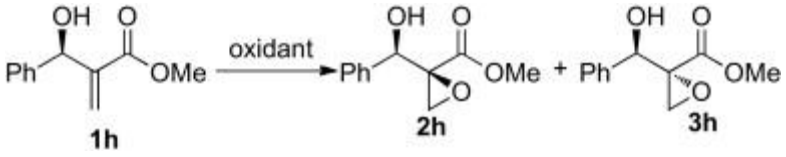


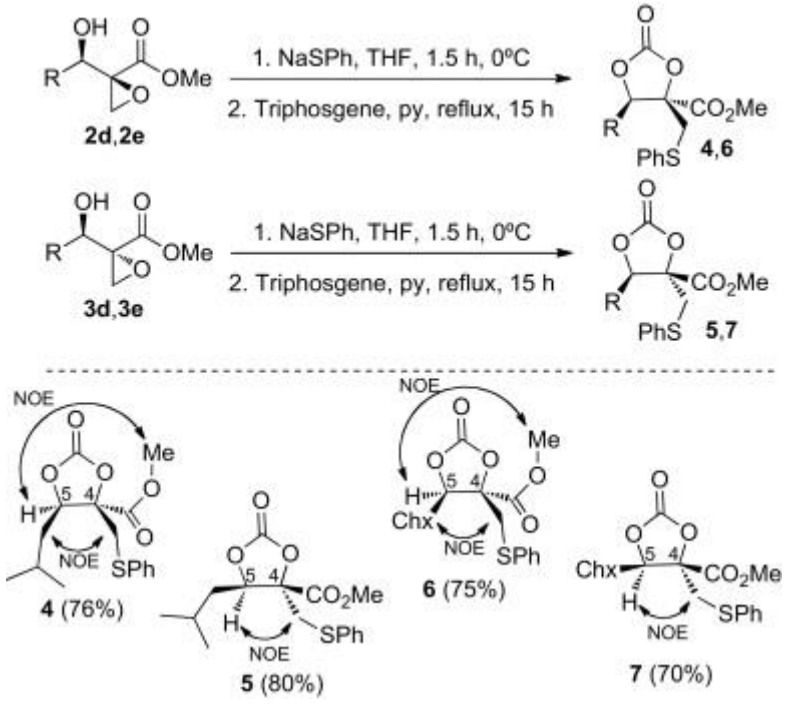

Table 3. Epoxidation of compound **1h**

| Entry | Conditions[a] | **2h**/**3h** | Yield (%)[b] |
|---|---|---|---|
| 1 | TBPLi | 88/12 | 66 |
| 2 | CMPLi | 91/9 | 72 |
| 3 | *m*-CPBA | 90/10 | 28 |
| 4 | *m*-CPBA | 88/12 | 80 |
| 5 | *m*-CPBA | — | NR |
| 6 | TBPNa | 85/15 | 41 |
| 7 | TBPNa | 87/13 | 62 |
| 8 | TBPK | 83/17 | 61 |

[a] For entry 1: 1.5 equiv of TBHP, 0.8 equiv of MeLi, THF, –20 °C, 20 h. For entry 2: 1.5 equiv of CMHP, 1.1 equiv of MeLi, THF, –20 °C, 20 h. For entry 3: 2.1 equiv of *m*-CPBA, DCM, rt, 96 h. For entry 4: 2.1 equiv of *m*-CPBA, 70 °C (sealed tube), 96 h. For entry 5: 2.5 equiv of *m*-CPBA, 1.3 equiv of $K_2CO_3$, DCM, rt, 96 h. For entry 6: 2.0 equiv of TBHP, 1.0 equiv of *t*-BuONa, THF, 0 °C, 3 h. For entry 7: 2.0 equiv of TBHP, 0.25 equiv of *t*-BuONa, THF, 0 °C, 3 h. For entry 8: 2.0 equiv of TBHP, 0.25 equiv of *t*-BuOK, THF, 0 °C, 3 h.

[b] Isolated yield of products corresponds to mixtures of *syn* and *anti* diastereomers.

The stereochemistry of epoxides **2b** and **h** was confirmed by comparison with already reported data.12 [and] 15 The epoxyesters **2d**, **3d**, **2e**, and **3e** were transformed into cyclic carbonates through a one-pot sequence: treatment with thiophenol in the presence of a base, which resulted in the opening of the oxirane ring and then addition of triphosgene to give carbonates **4**, **5**, **6**, and **7**, respectively (Scheme 3). The stereochemical assignment of the carbonates was performed by NOE experiments (Scheme 3). Carbonates **4** and **6**gave NOE between H-5 and methyl ester whilst **5** and **7** gave NOE between H-5 and methylene from the (phenylthio)methyl group.




Scheme 3. Cyclic carbonates **4**–**7**

In summary, the diastereoselectivity of the nucleophilic epoxidation of 3-hydroxy-2-methylene esters has been studied. The *syn* isomer was the major one in all cases. The resulting 3-hydroxy 2-epoxyesters were treated with thiophenol for transformation into 2,3-dihydroxy-2-((phenylthio)methyl), which upon treatment with triphosgene afforded the corresponding cyclic carbonates.

# Amidation through carbamates

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ABSTRACT

*N*-Alkyl carbamates of primary amines are easily converted into amides under treatment with Grignard reagents. Consequently, primary amines can be converted into amides in a one-pot reaction through carbamate protection and Grignard addition.



### 1. Introduction

Carbamates and amides are widely used protecting groups for amines.[1] While some recent reports have described the conversion of carbamates into amides,[2] more general and convenient methods to accomplish this transformation are desirable.

During the course to the attempted preparation of diphenyl-L-prolinol, we reacted *N*-CBZ-L-proline methyl ester[3] with phenyl magnesium bromide. Unexpectedly, we obtained *N*-benzoyl-L-prolinol **1** as the main product (Scheme 1).

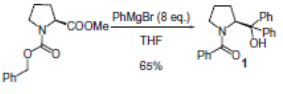
This result prompted us to examine the reactivity of carbamates with Grignard reagents. When carbamates of secondary amines are treated with Grignard reagents or organolithium compounds, the corresponding amine and ketone are obtained (Scheme 2).[4] Interestingly the reaction between carbamates of primary amines and Grignard reagents has been reported before as an undesired process.[5] We are pleased to report herein that carbamates of primary amines when treated with Grignard reagents gave rise to amides in high yield and that primary amines can be converted into amides in a one-pot reaction through carbamate protection and Grignard addition (Scheme 2).

### 2. Results

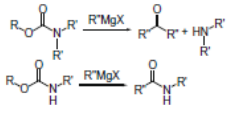
*N*-Alkyl carbamates (benzyl, methyl, and *terc*-butyl) of primary amines (benzylamine, allylamine, and 3-phenylpropylamine) were prepared according to standard procedures.[3] The resulting carbamates were reacted with Grignard reagents in THF at room temperature for 24 h (Table 1).

In all cases, starting carbamates were transformed into the corresponding amides[6] in good to high yield. The broad availability of Grignard reagents permitted the preparation of a wide variety of amides, which is an advantage over similar procedures in the literature.[2]

We are also glad to report a new one-pot procedure for the conversion of amines into amides. When a primary amine was treated with an alkoxy carbonyl chloride in the presence of triethyl amine, followed by addition of a Grignard reagent, the corresponding amide was obtained in good to high yield (Table 2).




**Scheme 1.** Treatment of *N*-CBZ-L-proline methyl ester with phenyl magnesium bromide.




**Scheme 2.** Treatment of carbamates of secondary and primary amines.

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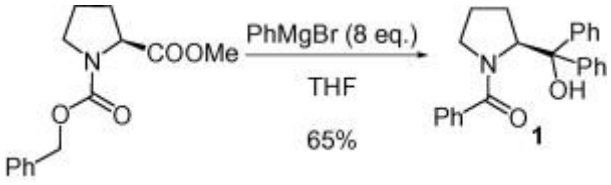
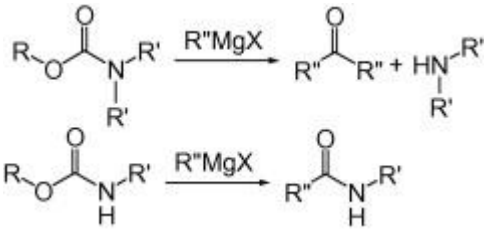


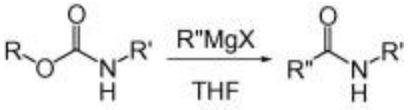

Scheme 1. Treatment of *N*-CBZ-L-proline methyl ester with phenyl magnesium bromide.

This result prompted us to examine the reactivity of carbamates with Grignard reagents. When carbamates of secondary amines are treated with Grignard reagents or organolithium compounds, the corresponding amine and ketone are obtained (Scheme 2).[4] Interestingly the reaction between carbamates of primary amines and Grignard reagents has been reported before as an undesired process.[5] We are pleased to report herein that carbamates of primary amines when treated with Grignard reagents gave rise to amides in high yield and that primary amines can be converted into amides in a one-pot reaction through carbamate protection and Grignard addition (Scheme 2).




Scheme 2. Treatment of carbamates of secondary and primary amines

*N*-Alkyl carbamates (benzyl, methyl, and *terc*-butyl) of primary amines (benzylamine, allylamine, and 3-phenylpropylamine) were prepared according to standard procedures.[1] The resulting carbamates were reacted with Grignard reagents in THF at room temperature for 24 h ( Table 1).



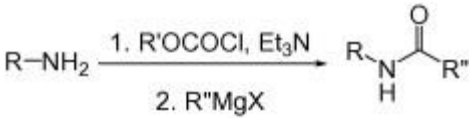

| Entry | R | R′ | Grignard (R″MgX) | Yield (%) |
|---|---|---|---|---|
| 1 | Bn | Bn | MeMgBr | 66 |
| 2 | Bn | Bn | EtMgBr | 70 |
| 3 | Bn | Bn | PhMgBr | 73 |
| 4 | Bn | Bn | *t*-BuMgCl | 74 |
| 5 | Bn | $PhCH_2CH_2CH_2$ | MeMgBr | 77 |
| 6 | Bn | $PhCH_2CH_2CH_2$ | EtMgBr | 72 |
| 7 | Bn | $PhCH_2CH_2CH_2$ | *t*-BuMgCl | 89 |
| 8 | Bn | $PhCH_2CH_2CH_2$ | *i*-PrMgCl | 62 |
| 9 | Bn | $CH_2{=}CHCH_2$ | MeMgBr | 43 |
| 10 | Bn | $CH_2{=}CHCH_2$ | EtMgBr | 43 |
| 11 | Bn | $CH_2{=}CHCH_2$ | *t*-BuMgCl | 69 |

| Entry | R | R′ | Grignard (R″MgX) | Yield (%) |
|---|---|---|---|---|
| 12 | Bn | $CH_2{=}CHCH_2$ | *i*-PrMgCl | 99 |
| 13 | Me | $PhCH_2CH_2CH_2$ | MeMgBr | 95 |
| 14 | Me | $PhCH_2CH_2CH_2$ | EtMgBr | 71 |
| 15 | Me | $PhCH_2CH_2CH_2$ | *t*-BuMgCl | 79 |
| 16 | Me | $PhCH_2CH_2CH_2$ | *i*-PrMgCl | 83 |
| 17 | *t*-Bu | $PhCH_2CH_2CH_2$ | EtMgBr | 85 |

Table 1. Treatment of carbamates with Grignard reagents

In all cases, starting carbamates were transformed into the corresponding amides[6] in good to high yield. The broad availability of Grignard reagents permitted the preparation of a wide variety of amides, which is an advantage over similar procedures in the literature.[2]

We are also glad to report a new one-pot procedure for the conversion of amines into amides. When a primary amine was treated with an alkoxy carbonyl chloride in the presence of triethyl amine, followed by addition of a Grignard reagent, the corresponding amide was obtained in good to high yield (Table 2).




| Entry | R | R′ | R″ | Yield (%) |
|---|---|---|---|---|
| 1 | $PhCH_2CH_2CH_2$ | Me | Et | 87 |
| 2 | $PhCH_2CH_2CH_2$ | Bn | Et | 92 |
| 3 | $PhCH_2CH_2CH_2$ | Bn | Me | 66 |

| Entry | R | R′ | R″ | Yield (%) |
|---|---|---|---|---|
| 4 | $CH_2{=}CHCH_2$ | Bn | Me | 70 |
| 5 | $CH_2{=}CHCH_2$ | Bn | *t*-Bu | 47 |
| 6 | $CH_2{=}CHCH_2$ | Bn | *i*-Pr | 58 |
| 7 | $CH_2{=}CHCH_2$ | Bn | Et | 36 |
| 8 | $CH_2{=}CHCH_2$ | Me | Et | 41 |
| 9 | $CH_2{=}CHCH_2$ | Me | Me | 83 |
| 10 | $PhCH_2CH_2CH_2$ | *t*-Bu | Et | 90 |

Table 2. One-pot conversion of amines into amides

This one-pot process represents a new approach to synthesizing amides from amines.

When carbamates of secondary amines are treated with Grignard reagents, the corresponding amines and ketones are obtained.[3] However, carbamates of primary amines give amides. Carbamates of primary amines probably react with Grignard reagents through an *N*,*O*-magnesium chelate **2** ( Fig. 1) whilst carbamates of secondary amines cannot form this intermediate. The unexpected formation of the amide of diphenyl-L-prolinol from *N*-CBZ-L-proline methyl ester could be explained by formation of chelate **3** (Fig. 1).

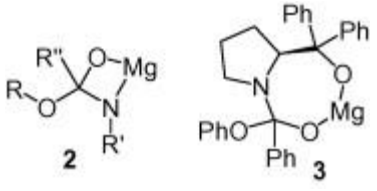



Figure 1. Chelation intermediates.

In summary, we have shown that carbamates of primary amines give rise to amides when treated with Grignard reagents. Primary amines can then be transformed into amides through protection as a carbamate followed by Grignard treatment in a one-pot procedure.

# Synthetic Studies on the Preparation of Alanyl Epoxysulfones as Cathepsin Cysteine Protease Electrophilic Traps

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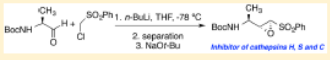
Supporting Information

ABSTRACT: A Darzens reaction between *tert*-butoxycarbonyl alaninal and chloromethyl phenyl sulfone afforded chlorohydrins, which were converted into epoxysulfones by reaction with sodium *tert*-butoxide. Epoxysulfone **10** and chloroketone **14** derived from chlorohydrins by oxidation proved to be inhibitors of cathepsins H, S, and C as determined by competitive activity-based protein profiling.




Lysosomal cysteine proteases (cathepsins) have been identified as therapeutic targets in the search for new drugs against a number of human pathologies, including cancer,[1] Alzheimer's disease,[2] and osteoporosis.[3] The design of new cathepsin inhibitors is intimately based on the discovery of new chemical moieties that react effectively and selectively with the cysteine thiol nucleophile present in the enzyme active site. Thiol-reactive groups present in cathepsin inhibitors include Michael acceptors, such as the vinyl sulfone K1777,[4] nitriles,[5] and azanitriles,[6] as well as electrophiles reactive toward $S_N2$ substitution. The most representative examples of the latter group are the epoxysuccinates,[7] as present for instance in the natural product E-64 and its derivatives,[8,9] halomethyl ketones[10] and acyloxymethyl ketones[11−13] (Figure 1). The here-studied epoxysulfone moiety is designed to combine structural features of two cysteine protease-reactive electrophiles: vinyl sulfones and epoxysuccinates.

In the context of our investigations of the preparation of new epoxide-based cathepsin inhibitors,[14] we became interested in the synthesis of epoxysulfones. The preparation of epoxysulfones derived from amino acids as new warheads of cysteine proteases was previously attempted through nucleophilic epoxidation of vinyl sulfones,[15] but it was reported that when vinyl sulfones were treated with lithium *tert*-butyl peroxide, allyl sulfones were formed instead of the desired epoxysulfones. We report herein the synthesis of epoxysulfones derived from amino acids through a Darzens reaction using amino aldehydes derived from the corresponding amino acids and chloromethyl phenyl sulfone.

In a first attempt, the Darzens reaction between *tert*-butoxycarbonyl alaninal (1 equiv) and chloromethyl phenyl sulfone (1 equiv) using 1 equiv of *n*-butyllithium in tetrahydrofuran as a solvent at low temperature (−78 °C) proved abortive in that the starting materials were recovered. After carrying out a set of experiments using increasing amounts of sulfone and base, we found that optimal results were obtained when 2.5 equiv of chloromethyl phenyl sulfone and 2.5 equiv of *n*-butyllithium were employed (Table 1, entry 1). In this case, a mixture of all four possible isomeric chlorohydrins **1**−**4** were obtained in good chemical yield. No significant changes were observed upon increasing the reaction time (entry 2) or performing the reaction at lower temperature (entry 3). This also held true when lithium diisopropylamide or lithium bis(trimethylsilyl)amide was used as the base instead of *n*-butyllithium (entries 4 and 5). In contrast, when dimethylformamide was added to the reaction mixture as a cosolvent, the *syn* isomer **3** was formed as the main reaction product (entry 6). On the other hand, when potassium *tert*-butoxide was used as the base under the same conditions as above, the *syn* isomers **3** and **4** were the main products isolated (entry 7). When the same reaction was performed at room temperature, a complex mixture of nonidentifiable products resulted, as observed by NMR spectroscopy of the crude reaction mixture.

Similar $J_{3,4}$ coupling constants were observed for *anti* chlorohydrins **1** and **2** and also for *syn* chlorohydrins **3** and **4**. For *anti* chlorohydrins **1** and **2**, $J_{3,4}$ was equal to 9.0 and 9.6 Hz respectively, while for *syn* chlorohydrins **3** and **4**, $J_{3,4}$ was equal to 0 Hz.

The mixture of four chlorohydrins **1**−**4** derived from the Darzens reaction was separated by silica gel chromatography. In order to establish the stereochemistries of the obtained chlorohydrins, they were transformed into oxazolidinones **6**−**9**. This transformation was attempted by initial deprotection the *tert*-butoxycarbonyl group followed by treatment with triphosgene (Scheme 1). When compound **1** was reacted with trifluoroacetic acid and the resulting ammonium salt was directly treated with triphosgene in the presence of triethylamine, unexpectedly chlorovinyl sulfone **5** was isolated as the single reaction product. Compound **5** results from N-deprotection and trifluoroacetamide formation followed by

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Lysosomal cysteine proteases (cathepsins) have been identified as therapeutic targets in the search for new drugs against a number of human pathologies, including cancer,(1) Alzheimer's disease,(2) and osteoporosis.(3) The design of new cathepsin inhibitors is intimately based on the discovery of new chemical moieties that react effectively and selectively with the cysteine thiol nucleophile present in the enzyme active site. Thiol-reactive groups present in cathepsin inhibitors include Michael acceptors, such as the vinyl sulfone K1777,(4) nitriles,(5) and azanitriles,(6) as well as electrophiles reactive toward $S_N2$ substitution. The most representative examples of the latter group are the epoxysuccinates,(7) as present for instance in the natural product E-64 and its derivatives,(8, 9) halomethyl ketones(10) and acyloxymethyl ketones(11-13) (Figure 1). The here-studied epoxysulfone moiety is designed to combine structural features of two cysteine protease-reactive electrophiles: vinyl sulfones and epoxysuccinates.

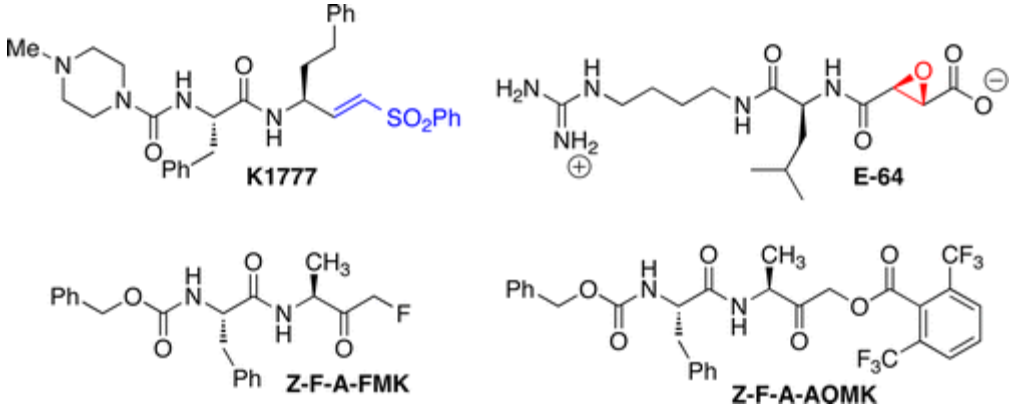
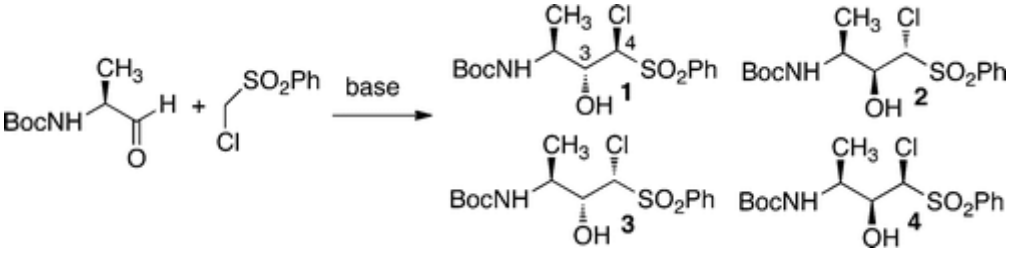



Figure 1. Known cysteine protease inhibitors.

In the context of our investigations of the preparation of new epoxide-based cathepsin inhibitors,(14) we became interested in the synthesis of epoxysulfones. The preparation of epoxysulfones derived from amino acids as new warheads of cysteine proteases was previously attempted through nucleophilic epoxidation of vinyl sulfones,(15) but it was reported that when vinyl sulfones were treated with lithium *tert*-butyl peroxide, allyl sulfones were formed instead of the desired

epoxysulfones. We report herein the synthesis of epoxysulfones derived from amino acids through a Darzens reaction using amino aldehydes derived from the corresponding amino acids and chloromethyl phenyl sulfone.

In a first attempt, the Darzens reaction between *tert*-butoxycarbonyl alaninal (1 equiv) and chloromethyl phenyl sulfone (1 equiv) using 1 equiv of *n*-butyllithium in tetrahydrofuran as a solvent at low temperature (–78 °C) proved abortive in that the starting materials were recovered. After carrying out a set of experiments using increasing amounts of sulfone and base, we found that optimal results were obtained when 2.5 equiv of chloromethyl phenyl sulfone and 2.5 equiv of *n*-butyllithium were employed (Table 1, entry 1). In this case, a mixture of all four possible isomeric chlorohydrins **1**–**4** were obtained in good chemical yield. No significant changes were observed upon increasing the reaction time (entry 2) or performing the reaction at lower temperature (entry 3). This also held true when lithium diisopropylamide or lithium bis(trimethylsilyl)amide was used as the base instead of *n*-butyllithium (entries 4 and 5). In contrast, when dimethylformamide was added to the reaction mixture as a cosolvent, the *syn*isomer **3** was formed as the main reaction product (entry 6). On the other hand, when potassium *tert*-butoxide was used as the base under the same conditions as above, the *syn*isomers **3** and **4** were the main products isolated (entry 7). When the same reaction was performed at room temperature, a complex mixture of nonidentifiable products resulted, as observed by NMR spectroscopy of the crude reaction mixture.

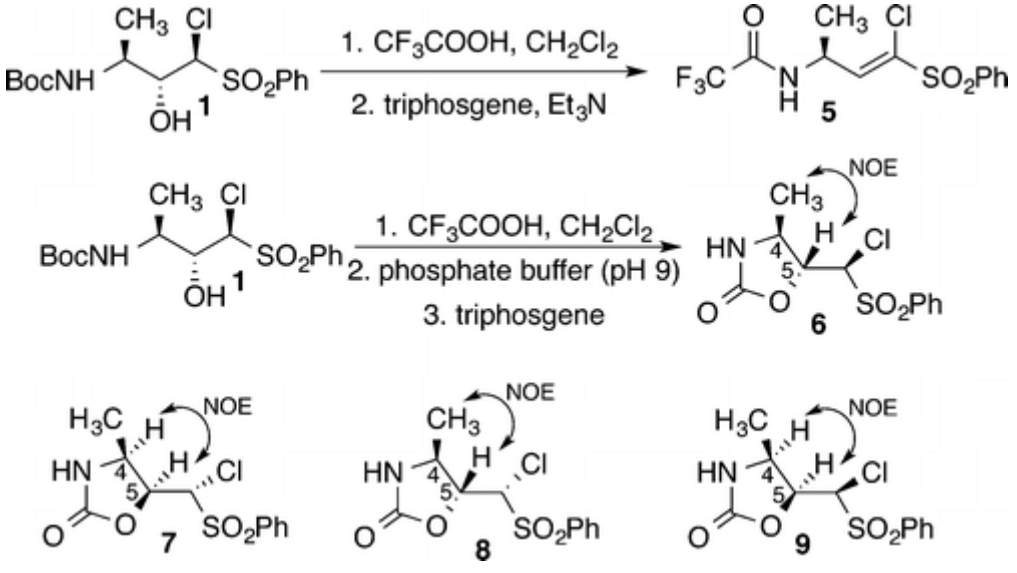


| Entry | Base | Conditions | 1:2:3:4 | Yield (%) |
|---|---|---|---|---|
| 1 | *n*-BuLi | THF, –78 °C, 30 min | 33:27:24:16 | 75 |
| 2 | *n*-BuLi | THF, –78 °C, 5 h | 35:26:25:14 | 77 |
| 3 | *n*-BuLi | THF, –90 °C, 2 h | 33:24:24:19 | 71 |
| 4 | LDA | THF, –78 °C, 30 min | 36:29:21:13 | 70 |
| 5 | LiHMDS | THF, –78 °C, 30 min | 35:25:25:15 | 67 |
| 6 | *n*-BuLi | THF/DMF, –78 °C, 30 min | 27:15:46:12 | 66 |
| 7 | *t*-BuOK | THF, –78 °C, 30 min | 17:8:42:33 | 55 |

Similar $J_{3,4}$ coupling constants were observed for *anti* chlorohydrins **1** and **2** and also for *syn*chlorohydrins **3** and **4**. For *anti* chlorohydrins **1** and **2**, $J_{3,4}$ was equal to 9.0 and 9.6 Hz respectively, while for *syn* chlorohydrins **3** and **4**, $J_{3,4}$ was equal to 0 Hz.

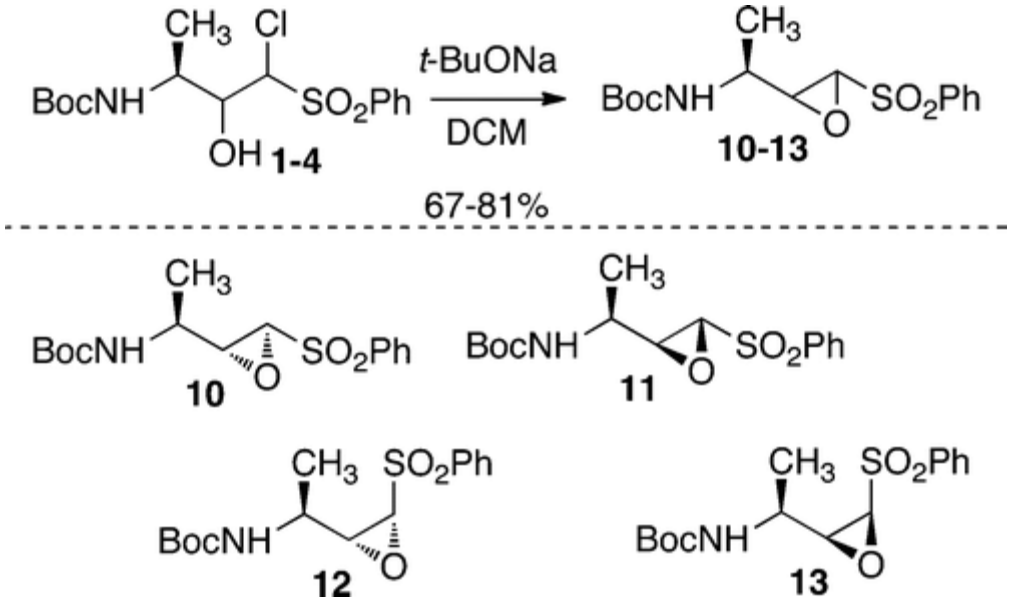
The mixture of four chlorohydrins **1**–**4** derived from the Darzens reaction was separated by silica gel chromatography. In order to establish the stereochemistries of the obtained chlorohydrins, they were transformed into oxazolidinones **6**–**9**. This transformation was attempted by initial deprotection the *tert*-butoxycarbonyl group followed by treatment with triphosgene (Scheme 1). When compound **1** was reacted with trifluoroacetic acid and the resulting ammonium salt was directly

treated with triphosgene in the presence of triethylamine, unexpectedly chlorovinyl sulfone **5** was isolated as the single reaction product. Compound **5** results from N-deprotection and trifluoroacetamide formation followed by elimination of water. In order to avoid the formation of such undesired compounds, the ammonium salt resulting from deprotection was transformed into the free amine with a phosphate buffer (pH 9.0), and then the free amine was converted into oxazolidinone **6** by reaction with triphosgene (Scheme 1). The same synthetic sequence was followed to prepare oxazolidinones **7**, **8**, and **9** starting from chlorohydrins **2**, **3**, and **4**, respectively. The configurations of the asymmetric carbon atoms in oxazolidinones **6**–**9** were established by NMR experiments (Scheme 1). Both oxazolidinones **6** and **8** exhibited NOEs between H-5 and the methyl protons, while oxazolidinones **7** and **9** exhibited NOEs between H-5 and H-4.



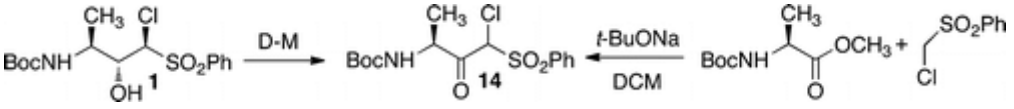

Scheme 1. Transformation of Chlorohydrins into Oxazolidinones

An extensive experimental investigation was then carried out to convert chlorohydrins into epoxysulfones by base treatment. First, chlorohydrin **1** was treated with sodium hydride, but NMR spectra of the crude mixture showed starting material, isomer **3** resulting from epimerization, and, as major products, alaninal and chloromethyl sulfone resulting from a retro-Darzens reaction. When chlorohydrin **2** was treated with sodium hydride, conversion into the isomeric compound **4** and retro-Darzens products was also observed. Triethylamine did not afford the desired compounds. Potassium carbonate in different solvents was next attempted. When methanol was used, the epoxide was detected as a minor product, and mainly starting material was recovered. When tetrahydrofuran was used as the solvent, traces of epoxide were detected, but epimerization to give compound **3** was also observed. Potassium carbonate in dimethylformamide gave the desired epoxide, but the retro-Darzens reaction also took place. Potassium *tert*-butoxide was then tried. When it was used in tetrahydrofuran as the solvent, the retro-Darzens reaction took place. Finally, sodium *tert*-butoxide gave *trans*-epoxysulfone **10** as the only reaction product when dichloromethane was applied as the solvent. In the case of *cis*-epoxysulfones, a mixture of dichloromethane and *tert*-butanol was used as a solvent. Under these reaction conditions, *trans*-epoxide **11** and *cis*-epoxides **12** and **13** were obtained from compounds **2**, **3**, and **4** respectively (Scheme 2).




Scheme 2. Transformation of Chlorohydrins into Epoxysulfones

Chlorohydrin **1** was transformed into chloroketone **14** through oxidation. Compound **14** was also prepared by addition of chloromethyl phenyl sulfone to N-protected methyl L-alaninate (Scheme3). Chloroketone **14** was obtained in both cases as an equimolar mixture of epimers.




Scheme 3. Preparation of Chloroketone 14

Compounds **10** and **14** were investigated for their ability to inhibit cathepsins by using a competitive activity-based protein profiling (ABPP) assay against the known cysteine protease cathepsin activity-based probe DCG04-BodipyFL.(16) Proteins from mouse liver lysates at pH 5.0 were treated with

compounds **10** and **14** for 1 h at 37 °C, and the residual cathepsin activity was captured by the DCG04-BodipyFL probe. After SDS-PAGE separation and in-gel fluorescence imaging, a typical band pattern was seen, which was compared with reported activity-based cathepsin profiling gels.(17) By comparison of the molecular weights of the individual bands seen, we have assigned the nature of the individual cathepsins labeled by DCG04-BodipyFL as indicated in Figure 2. Competition for most of the bands was observed upon cotreatment with DCG04-BodipyFL, a result that underscores the annotation of the bands as being DCG04-BodipyFL-sensitive cathepsins. Compound **10** is a more selective inhibitor than DCG04-BodipyFL, as it inhibits one to three cathepsins, while compound **14** inhibits four cathepsins at the 1 μM concentration used in this assay. Compounds **10** and **14** are able to outcompete the DCG04-BodipyFL inhibitor, and we can conclude that they bind in a covalent and irreversible fashion to the cathepsins, inhibiting their activity.

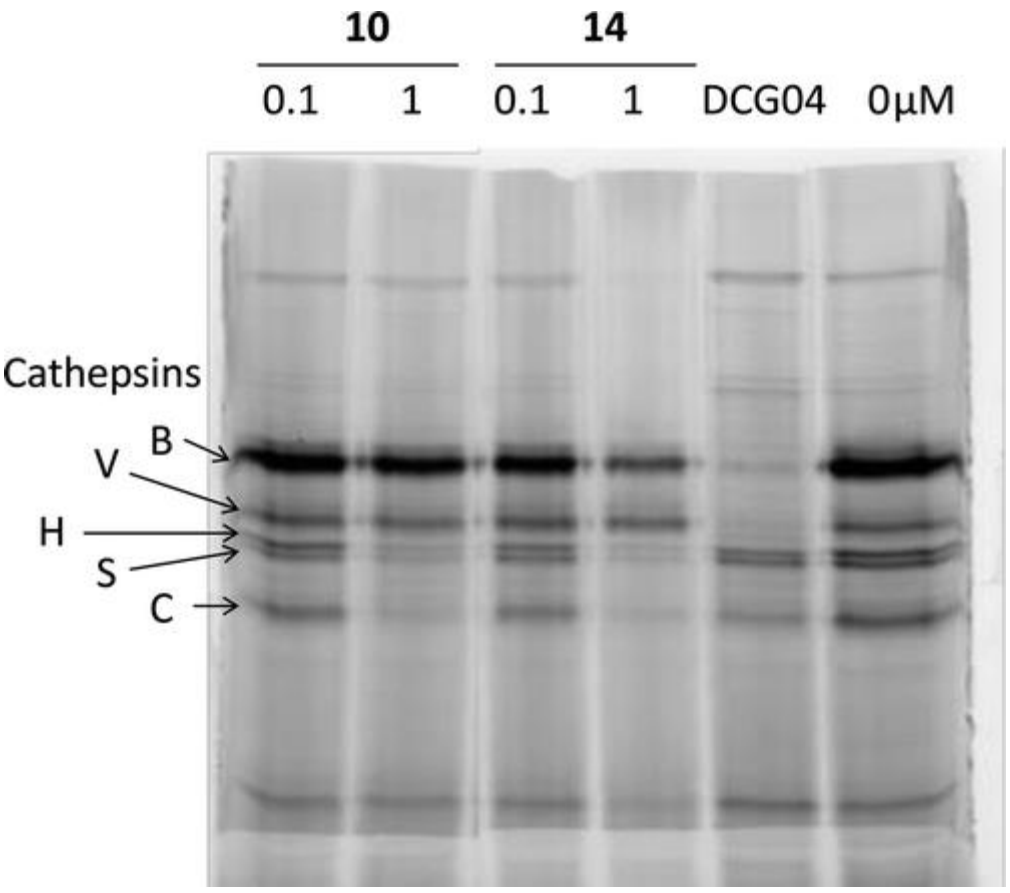



Figure 2. Competitive activity-based protein profiling (ABPP) assay of compounds 10 and 14 against the DCG04-Bodipy-FL probe in mouse liver lysate at pH 5.0. The inhibitors were used at 0.1 and 1 μM concentration, and the cathepsin probe was used at 0.5 μM. DMSO (1 μL) was used as negative control (0).

In summary, the Darzens reaction between *tert*-butoxycarbonyl alaninal and chloromethyl phenyl sulfone afforded chlorohydrins, which were converted into epoxysulfones by reaction with sodium *tert*-butoxide. Epoxysulfone **10** and chloroketone **14** derived from chlorohydrins by oxidation proved to be inhibitors of cathepsins H, S, and C, as determined by competitive activity-based protein profiling. The introduction of epoxysulfone warheads, including those obtained

from further amino acid-derived amino aldehydes, into more elaborate peptidic sequences may result in more specific and/or more active cysteine protease inhibitors, whereas introduction of a reporter molecule (as in DCG-04) may yield a new class of activity-based cysteine protease probes.

# Brief Curriculum Vitae

Antonio Latorre Martínez was born in Nules (Spain) in 1984. He obtained his high school degree at the Salesianos School in Burriana (Castellón). In 2007 he obtained his Chemistry degree with first honors from University Jaume I at Castellón.

The author of this thesis started his PhD research in December 2007 in the Medical and Pharmacological Chemistry Research Group at the same University under the guidance of Dr. Florenci Gonzalez Adelantado and Dr. Santiago Rodriguez Pastor and worked there until 2015. In 2009 he obtained his Advanced Studies Degree and the Master in Secondary Education. In 2012, as a part of his PhD training, he carried out a research stay at Leiden University under the supervision of Dr. Hermen Overkleeft designing and testing cysteine protease inhibitors.

Antonio Latorre Martínez

PhD THESIS 2015

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